EPA Registration Jacket 74234-EUP-E

ISB'S Front-end PRIA Completeness Screen

Draft 3; 10/25/07

EPA Receipt Date: JUN 2 3 2009		EPA Reg. Number: 74234-EUP-E		
	Check List Item	Yes	No	N/A
1	Has the **PRIA Fee been Paid**; is a copy of the check or Pay.gov receipt included in the Submission Package? (FW)	X		
2	Is an **Application Form** (EPA Form 8570-1) Included in the Submission Package, is it completely filled out and signed including package type?	X		
3	Is a **Confidential Statement of Formula** (EPA Form 8570-29) Included in the Submission Package, is it completely filled out and signed (boxes 1-21)?	X		
4	Is a **Formulator's Exemption Statement** (EPA Form 8570-27) Included in the Submission Package?		X	
5	Is a **Certification with Respect to Citation of Data** (EPA Form 8570-34) Included in the Submission Package?	X		
6	Is a **Data Matrix** (EPA Form 8570-35) Included in the Submission Package?	X		
7	Is a **Label** Included in the Submission Package?		X	
8	Are **Data** Included in the Submission Package?	X		
9	**Is the Submission an Amendment?**		X	

due 4/13

Experimental Use Permit

74234 – EUP-E

Bacteriophage [this one: E. coli]

a virus that attacks bacteria

naturally occurring

used as early as 1920's for medicinal purposes

(FDA has approved direct food additive of bacteriophages to lunch meat)

This one bacteriophage is used to treat food handling equip in food processing plants – residue will occur – tempory tolerance

Material Sent for Data Extraction

Reg # 74234-EUP-2

Description: experimental use permit

☒ Material(s) Sent to Data Extraction Contractors:

- ☐ New Stamped Label Dated _____
- ☐ Notification Dated _____
- ☐ New CSF(s) Dated _____
- ☒ Other: EUP stamped label

☒ Decision #: 416027

☐ Other Action/Comments: _____

Attach this coversheet to the top of the material or jacket. It must be well organized and clipped together, NOT STAPLED. Then give the material with this coversheet to staff in the Information Services Center (Room S-4900).

Reviewer: Tracy Lantz

Phone: 703 308 6415 Division: AD

Date: 4/7/11

Created February 3, 2011

UNITED ATES ENVIRONMENTAL PROTECTION ENCY

APR - 5 2011

Eliot Harrison
Agent for Intralytix, Inc.
122 C Street, N.W., Suite 740
Washington, D.C. 20001

Subject: ECP-100
EPA Experimental Use Permit No. 74234-EUP-2
Application date: May 30, 2009
Effective Dates: April 5, 2011 through April 1, 2013
Quantity authorized for application: 120,000 pounds of formulated product (0.392 pounds active ingredient)

Dear Mr. Harrison,

On the basis of the information furnished by the applicant, an Experimental Use Permit (EUP) under Section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (86 Stat. 983), is hereby issued for the new active ingredient *Escherichia coli* O157:H7 Specific Bacteriophages which is a bacterial virus specific against *Escherichia coli* O157:H7 to investigate the efficacy of the product on food and non-food contact surfaces via a field study. This product is only to be used as an adjuvant with and prior to the application of EPA registered food contact sanitizers.

Prior to shipment and/or use of this material, you must consult with the State [Pesticide Regulatory Officials of the States in which your experimental program will be conducted and obtain a state permit or license if such is required. Issuance of this federal permit does not negate the need for permission from the individual states. Failure to do so may result in revocation or modification of this experimental use permit.

CONCURRENCES								
SYMBOL	7510P							
SURNAME	S Funk							
DATE	4/5/11							

Shipment and/or use under this permit are subject to the provisions of 40 CFR §172. Based upon the experimental program, this product may be shipped for use under this permit to Nebraska, Washington, Texas, Kansas, Iowa and Illinois to beef processing plants operated by Tyson Fresh Meats, Inc.

You must comply with 40 CFR §172.8(b)(2), which states: "A final report shall be submitted within 180 days after the expiration of the permit, unless a request for extension of time is approved, and shall include: (i) all data gathered during the testing program (field notes need not be submitted but must be maintained, and submitted upon request), and (ii) a description of the disposition of any pesticide containers and any unused pesticide (including amounts disposed of and the method and site of disposition)."

All bacteriophage host strains used for production must be confirmed shigatoxin free in a manner similar to the analyses used for *E. coli* Ec211/ECOR-56/ATCC 35375 (MRIDs 4815201 and 48152102).

The labeling submitted in connection with the application for an experimental use permit (EUP) is acceptable, subject to the following conditions. Revise your labeling as follows:

1. "EPA Experimental Use Permit No. 74234-EUP-2" must appear on the label.
2. Add the following statement to the directions for use: "*Gross filth or heavy soil should be removed before application of this product.*" Please note that in addition, if you do not add a pre-cleaning step prior to product application, when developing your efficacy data you will need to incorporate a soil load.
3. Revise the directions for use to include mandatory language as per PR Notice 2000-5 as follows: "ECP-100 *is for use* on food and non-food..."
4. Provide clarity to the directions for use as follows: "...Apply ECP-100 at least 5 minutes prior to using an EPA registered *sanitizer. Then follow the directions for use on the* EPA registered sanitizer."
5. Revise the Container Disposal language (both sections) such that the language is appropriate for a public health pesticide product. Revise the 5 gallon and less statement to include: "...Shake for 10 seconds. *Store rinsate for disposal.* Drain for 10 seconds..." Revise the greater than 5 gallons statement to include: "...Turn the container over onto its other end and tip it back and forth several times. *Store rinsate for disposal.* Repeat this procedure..."

CONCURRENCES								
SYMBOL								
SURNAME								
DATE								

A stamped copy of the label is enclosed for your records. This labeling must be used for all shipments of this product under the subject EUP. Submit one copy of the revised labeling for our records. Should you have any questions concerning this letter, please contact Tracy Lantz at (703) 308-6415.

Sincerely,

Dennis H Edwards Jr

Dennis Edwards, Jr.
Branch Chief
Regulatory Management Branch I
Antimicrobials Division (7510P)

Enclosure: Stamped Label
7510P:T.Lantz:4/4/11:EUP letter Intralytix ECP-100

CONCURRENCES								
SYMBOL								
SURNAME								
DATE								

Directions for Use

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

ECP-100 can is for use on food and non-food-contact surfaces in food-processing plants. Prior to application, add 1 part of ECP-100 into a clean container. Then add 9 parts of non-chlorinated water. If water is taken from a chlorinated source, allow the water to sit at room temperature for 24 hours prior to addition to ECP-100. After dilution, the use-solution or working titer of ECP-100 is approximately 10^9 PFU/ml. Apply the ECP-100 use-solution by either spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to ECP-100 application.

Only use ECP-100 as an adjunct to EPA registered food-contact surface sanitizers. Apply ECP-100 at least 5 minutes prior to using an EPA registered sanitizer following the use-instructions for the EPA registered sanitizer.

Precautionary Statements

Hazards to Humans: Avoid contact with eyes, skin or clothing. Wash thoroughly with soap and water after handling.

FOR EXPERIMENTAL-USE ONLY
ECP-100

For the control of *E. coli* 0157:H7 on Food and Non-Food Contact Surfaces in Food Processing Plants

NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT OR COOPERATOR OF THE EPA-APPROVED EXPERIMENTAL USE PROGRAM

Active Ingredient	
E. coli 0157:H7 Specific Lytic Bacteriophages*	0.00027%
Inert Ingredients	99.99973%
Total	100.0%

* Nominal titer of ECP-100 is 10^{10} PFU/ml

KEEP OUT OF REACH OF CHILDREN

CAUTION

EPA Experimental Use Permit No. 74234-EUP-
EPA Establishment Number:

ACCEPTED
for shipment and use of product for experimental purposes under the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act.
74234-EUP-2
Issued on 4/5/11

Net Contents:

Intralytix Inc.
701 East Pratt St.
Baltimore, MD 21202

Expiration Date: (60 days from the date of manufacture will be inserted)

Storage and Disposal

Do not contaminate water, food or feed by storage or disposal.

Storage: Store in original plastic container at 4°C.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on-site or at an approved waste disposal facility.

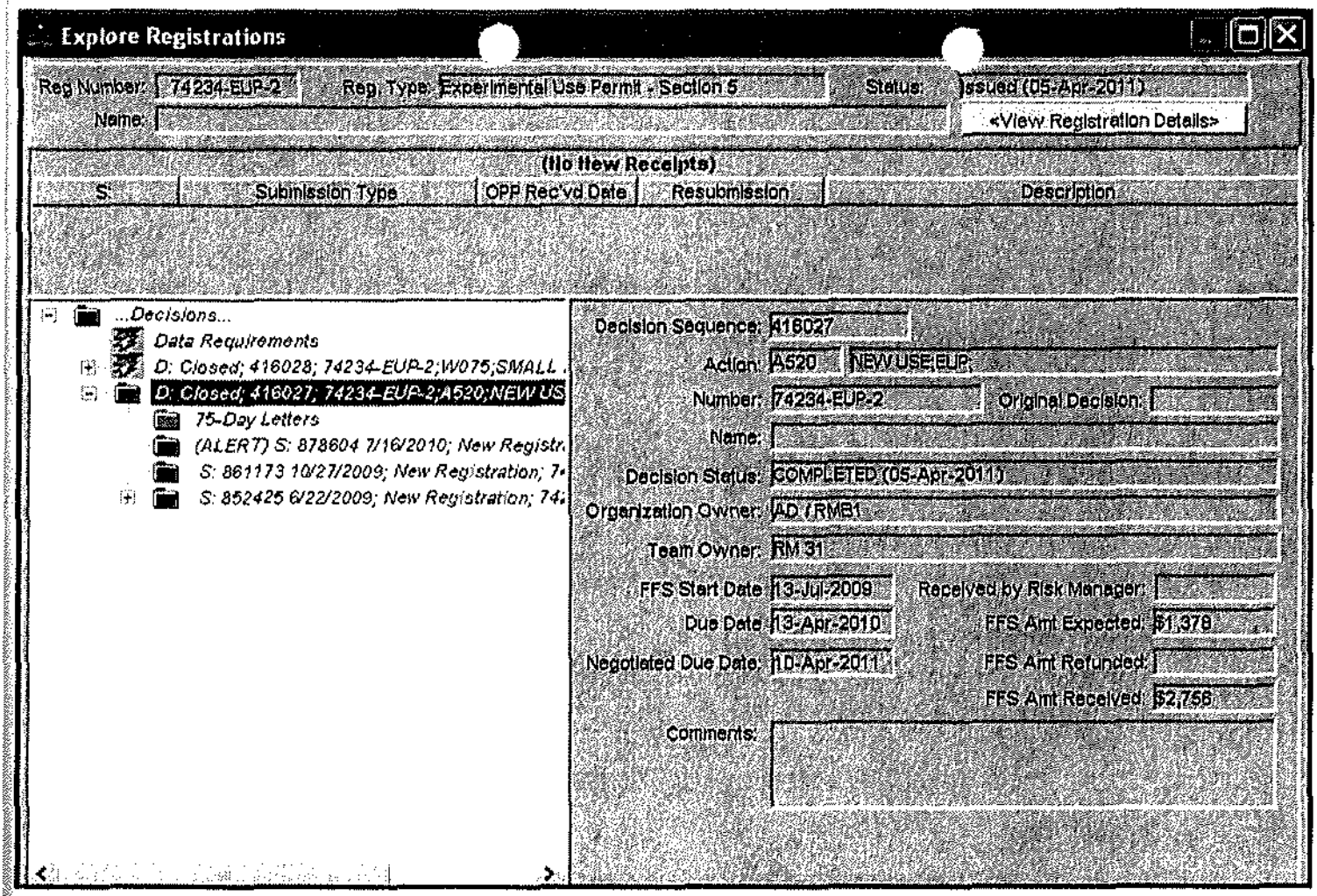
[For plastic containers equal to or less than 5 gallons].

Container Disposal: Nonrefillable container. Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container ¼ full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Then offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.

[For plastic containers more than 5 gallons].

Container Disposal: Nonrefillable container. Do not reuse or refill this container. Triple rinse container (or equivalent) promptly after emptying. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank. Fill the container ¼ full with water. Replace and tighten closures. Tip container on its side and roll it back and forth, ensuring at least one complete revolution, for 30 seconds. Stand the container on its end and tip it back and forth several times. Turn the container over onto its other end and tip it back and forth several times. Empty the rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Repeat this procedure two more times. Then offer for recycling if available or

puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke.


Explore Registrations
Reg Number: 74234-EUP-2
Reg. Type: Experimental Use Permit - Section 5
Status: Issued (05-Apr-2011)
Name:
«View Registration Details»
(No New Receipts)
S:
Submission Type
OPP Rec'vd Date
Resubmission
Description
...Decisions...
Data Requirements
D: Closed; 416028; 74234-EUP-2;W075;SMALL
D: Closed; 416027; 74234-EUP-2;A520;NEW US
75-Day Letters
(ALERT) S: 878604 7/16/2010; New Registr
S: 861173 10/27/2009; New Registration; 7
S: 852425 6/22/2009; New Registration; 74
Decision Sequence: 416027
Action: A520 NEW USE EUP
Number: 74234-EUP-2
Original Decision:
Name:
Decision Status: COMPLETED (05-Apr-2011)
Organization Owner: AD (RMB1
Team Owner: RM 31
FFS Start Date: 13-Jul-2009
Received by Risk Manager:
Due Date: 13-Apr-2010
FFS Amt Expected: $1,378
Negotiated Due Date: 10-Apr-2011
FFS Amt Refunded:
FFS Amt Received: $2,756
Comments:

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Certification with Respect to Citation of Data

Applicant's/Registrant's Name, Address and Telephone Number **Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201**	EPA Registration Number/ File Symbol **74234-**
Active Ingredient(s) and/or representative test compound(s): **E. coli 0157:H7 Specific Bacteriophages**	Date **May 30, 2009**
General use pattern(s) (list all those claimed for this product using 40 CFR Part 158) Indoor – Food and Non-Food	Product Name **ECP-100**

NOTE: If your product is a 100% repackaging of another purchased EPA-registered product labeled for all the same uses on your label, you do not need to submit this form. You must submit the Formulator's Exemption Statement (EPA Form 8570-27).

☐ I am responding to a Data Call-In Notice, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).

SECTION I: METHOD OF DATA SUPPORT (Check one method only)

☐ I am using the cite-all method of support, and have included with this form a list of companies sent offers of compensation (the Data Matrix form should be used for this purpose).	☒ I am using the selective method of support (or cite-all option under the selective method), and have included with this form a completed list of data requirements (the Data Matrix form must be used).

SECTION II: GENERAL OFFER TO PAY

[Required if using the cite-all method or when using the cite-all option under the selective method to satisfy one or more data requirements]

☐ I hereby offer and agree to pay compensation, to other persons, with regard to the approval of this application, to the extent required by FIFRA.

SECTION III: CERTIFICATION

I certify that this application for registration, this form for reregistration, or this Data Call-In Notice is supported by all data submitted or cited in the application for registration, the form for reregistration, or this Data Call-In response. In addition, if cite-all option under the selective method is indicated in Section I, this application is supported by all data in the Agency's files that (1) concern the properties or effects of this product or an identical or substantially similar product, or one or more of the ingredients in this product; and (2) is a type of data that would be required to be submitted under the data requirements in effect on the date of approval of this application if the application sought the initial registration of a product of identical or similar composition and uses.

I certify that for each exclusive use study cited in support of this registration or reregistration, that I am the original submitter or that I have obtained the written permission of the original submitter to cite that study.

I certify that for each study cited in support of this registration or reregistration that is not an exclusive use study, either: (a) I am the original data submitter; (b) I have obtained the written permission of the original data submitter to use this study in support of this application; (c) all periods of eligibility for compensation have expired for the study; (d) the study is in the public literature; or (e) I have notified in writing the company that submitted the study and have offered (i) to pay compensation to the extent required by sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA; and (ii) to commence negotiations to determine the amount and terms of compensation, if any, to be paid for the use of the study.

I certify that in all instances where an offer of compensation is required, copies of all offers to pay compensation and evidence of their delivery in accordance with sections 3(c)(1)(F) and/or 3(c)(2)(B) of FIFRA are available and will be submitted to the agency upon request. Should I fail to produce such evidence to the Agency upon request, I understand that the Agency may initiate action to deny, cancel or suspend the registration of my product in conformity with FIFRA.

I certify that the statements I have made on this form and all attachments to it are true, accurate and complete. I acknowledge that any knowingly false or misleading statements may be punishable by fine or imprisonment or both under applicable law.

Signature	Date	Typed or Printed Name and Title
	5/30/09	Eliot Harrison, Agent for Intralytix, Inc.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

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DATA MATRIX

Date 4/05/20 f f

EPA Reg. No./File Symbol 74234-EUP-E

Applicant's/Registrant's Name & Address:
Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201

Product
ECP-100™

Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
PRODUCT ANALYSIS					
885.1100	Product Identity	47786801 X	Intralytix, Inc.	OWN	
885.1200	Manufacturing Process	47786801 X	Intralytix, Inc.	OWN	
885.1300	Deposition of Sample in Culture Collection and Discussion of Formation of Impurities	47786801 X	Intralytix, Inc.	OWN	
885.1400	Analysis of Samples	47786802 X 48152101 X 48152102 X	Intralytix, Inc Intralytix, Inc. Intralytix, Inc.	OWN OWN OWN	
885.1500	Certification of Limits	47786802 X	Intralytx, Inc.	OWN	
830.6302	Color	47786802 X	Intralytix, Inc.	OWN	
830.6303	Physical State	47786802 X	Intralytix, Inc.	OWN	
830.6304	Odor	47786802 X	Intralytix, Inc.	OWN	
830.6313	Stability	47786802 X	Intralytix, Inc.	OWN	
830.6317	Storage Stability	47786802 X	Intralytix, Inc.	OWN	
830.6319	Miscibilty	47786802 X	------	----	Footnote t
830.6320	Corrosion Characteristics	47786802 X	Intralytix, Inc.	OWN	
830.7000	pH	47786802 X	Intralytix, Inc.	OWN	
830.7100	Viscosity	47786802 X	Intralytix, Inc.	OWN	
830.7300	Density	47786802 X	Intralytix, Inc.	OWN	
TOXICOLOGY					
885.3050	Acute Oral Toxicity/Pathogenicity	Waiver Request	-----	----	Footnote 2
885.3150	Acute Pulmonary Toxicity/Pathogenicity	Waiver Request	----	----	Footnote 2
885.3200	Acute Injection Toxicity/Pathogenicity	Waiver Request	-----	-----	Footnote 2
885.3400	Hypersensitivity Incidents	----	----	----	Footnote 3
885.3500	Cell Culture	Waiver Request	----	-----	Footnote 2
870.1100	Acute Oral Toxicity	Waiver Request	-----	-----	Footnote 2
870.1200	Acute Dermal Toxicity	Waiver Request	-----	-----	Footnote 2
870.1300	Acute Inhalation Toxicity	Waiver Request	------	------	Footnote 2
870.2400	Acute Eye Irritation	Waiver Request	-----	------	Footnote 2

Signature	Name and Title:	Date
Eliot	Eliot Harrison, Agent for Intralytix, Inc.	4/05/2011

EPA Form 8570-35 [9-97] Electronic and Paper versions available. Submit only Paper version.

Agency Internal Use Copy

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

DATA MATRIX

Date 4/05/2011	EPA Reg. No./File Symbol 74234-EUP-E	Page 2 of 3
Applicant's/Registrant's Name & Address: Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201	Product ECP-100™	
Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages		

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
870.2500	Primary Dermal Irritation	Waiver Request	-----	-----	Footnote 2
NON-TARGET ORGANISM AND ENVIRONMENTAL EXPRESSION					
885.4050	Avian Oral LD50	N/A	-----	-----	Footnote 4
885.4200	Acute Freshwater Fish	N/A	-----	-----	Footnote 4
885.4240	Freshwater – Aquatic Invertebrates	N/A	-----	-----	Footnote 4
885.4300	Non-Target Plants	N/A	-----	-----	Footnote 4
885.4340	Non-Target Insects	N/A	-----	-----	Footnote 4
885.4380	Honey Bee Toxicity	N/A	-----	-----	Footnote 4
RESIDUE					
885.2100	Chemical Identity	N/A	-----	-----	Footnote 5
885.2200	Nature of the Residue in Plants	N/A	-----	-----	Footnote 5
885.2250	Nature of the Residue in Animals	N/A	-----	-----	Footnote 5
885.2300	Analytical Methods – Plants	N/A	-----	-----	Footnote 5
885.2350	Analytical Methods – Animals	N/A	-----	-----	Footnote 5
885.2400	Storage Stability	N/A	-----	-----	Footnote 5
885.2500	Magnitude of the Residue in Plants	N/A	-----	-----	Footnote 5
885.2550	Magnitude of the Residue in Meat, Milk, Poultry, Eggs	N/A	-----	-----	Footnote 5
885.2600	Magnitude of the Residue in Potable Water, Fish, and Irrigated Crops	N/A	-----	-----	Footnote 5
EFFICACY					
91-2	Products for Use on Hard Surfaces	47893701 X	Intralytix, Inc.		

Signature	Name and Title:	Date
Eliot	Eliot Harrison, Agent for Intralytix, Inc.	4/05/2011

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

DATA MATRIX

Date 4/05/2011	EPA Reg. No./File Symbol 74234-EUP-E	Page 3 of 3
Applicant's/Registrant's Name & Address: Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201	Product ECP-100™	
Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages		

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note

FOOTNOTES:

1. The miscibility data requirement is not applicable since the finished product, ECP100, is not mixed with petroleum solvents.
2. Waiver requests for these studies have previously been submitted to the Agency.
3. Hypersensitivity incidents will be reported if they occur.
4. All of the Non-Target Organism and Environmental Fate data requirements are not applicable since the experimental use is indoors.
5. All of the Residue data requirements are not applicable since the microbial pesticide, *E. coli* 0157:H7 specific bacteriophages does not have the potential to cause adverse human health effects.

Signature	Name and Title: Eliot Harrison, Agent for Intralytix, Inc.	Date 4/05/2011

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

DATA MATRIX

Date 4/05/2011	EPA Reg. No./File Symbol 74234-EUP-E	Page 1 of 3
Applicant's/Registrant's Name & Address: Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201	Product ECP-100™	

Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
PRODUCT ANALYSIS					
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc Intralytix, Inc. Intralytix, Inc.	OWN OWN OWN	
			Intralytx, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			----	---	Footnote 1
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			Intralytix, Inc.	OWN	
			----	----	Footnote 2
			----	----	Footnote 2
			----	----	Footnote 2
			----	----	Footnote 3
			----	----	Footnote 2
			----	----	Footnote 2
			----	----	Footnote 2
			----	----	Footnote 2
			----	----	Footnote 2

Signature	Name and Title: Eliot Harrison, Agent for Intralytix, Inc.	Date 4/05/2011

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

DATA MATRIX

Date 4/05/2011	EPA Reg. No./File Symbol 74234-EUP-E	Page 2 of 3
Applicant's/Registrant's Name & Address: Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201	Product ECP-100™	

Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
870.2500	Primary Dermal Irritation	Waiver Request	---	---	Footnote 2
NON-TARGET ORGANISM AND ENVIRONMENTAL EXPRESSION					
			---	---	Footnote 4
			---	---	Footnote 4
			---	---	Footnote 4
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			---	---	Footnote 5
			---	---	Footnote 5
			---	---	Footnote 5
			---	---	Footnote 5
			---	---	Footnote 5
			Intralytix, Inc.		

Signature	Name and Title:	Date
Elliot	Elliot Harrison, Agent for Intralytix, Inc.	4/05/2011

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
401 M Street, S.W.
WASHINGTON, D.C. 20460

Form Approved OMB No. 2070-0060

DATA MATRIX

Date 4/05/2011	EPA Reg. No./File Symbol 74234-EUP-E	Page 3 of 3
Applicant's/Registrant's Name & Address: Intralytix, Inc., 323 West Camden Street, Suite 675, Baltimore, MD 21201	Product ECP-100™	

Ingredient(s): *E. coli* 0157:H7 Specific Lytic Bacteriophages

Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note

FOOTNOTES:

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5. All of the Residue data requirements are not applicable since the microbial pesticide, *E. coli* 0157:H7 specific bacteriophages does not have the potential to cause adverse human health effects.

Signature	Name and Title: Eliot Harrison, Agent for Intralytix, Inc.	Date 4/05/2011

Study Information For
Experimental Use Permit - Section 5
74234-EUP-E

MRID	Citation	Receipt Date
47786800	Intralytix, Inc. (2009) Submission of Product Chemistry and Toxicity Data in Support of the Petition for Tolerance and Experimental Use of E.Coli 0157:H7 Specific Bacteriophages. Transmittal of 3 Studies.	22-Jun-2009
47786801	Harrison, E. (2009) ECP-100-Product Identity, Manufacturing Process, Sample Deposition and Discussion of Fomation of Impurities. Project Number: ECP/100/PC001. Unpublished study prepared by Intralytix, Inc. 33 p.	22-Jun-2009
47786802	Harrison, E. (2009) ECP-100 - Analysis of Samples, Certification of Limits and Physical and Chemical Characteristics. Project Number: ECP/100/PC001. Unpublished study prepared by Intralytix, Inc. 13 p.	22-Jun-2009
47786803	Harrison, E. (2009) Waiver Requests for Microbial Pesticide Toxicology Data Requirements and Discussion of Safety Issues. Project Number: ECP/100/SA001. Unpublished study prepared by Intralytix, Inc. 231 p.	22-Jun-2009
47893700	Intralytix, Inc. (2009) Submission of Efficacy Data in Support of the Experimental Use of ECP-100. Transmittal of 1 Study.	27-Oct-2009
47893701	Sulakvelidze, A. (2009) ECP-100: Efficacy Information. Project Number: M001/6. Unpublished study prepared by Intralytix, Inc. 8 p.	27-Oct-2009
48152100	Intralytix, Inc. (2010) Submission of Efficacy Data in Support of the Experimental Use of ECP-100. Transmittal of 2 Studies.	16-Jul-2010
48152101	Carter, C. (2010) Test for the Presence of Shiga Toxin Genes Stx-1 and Stx-2 in Ec211, or ATCC 35375. Project Number: CDC051710. Unpublished study prepared by Intralytix, Inc. 19 p.	16-Jul-2010
48152102	Sulakvedlidze, A. (2010) Lytic Activity of Component Monophages. Unpublished study prepared by Intralytix, Inc. 6 p.	16-Jul-2010
Total Rows: 9		

1) What is the hypersensitivity study (870.2600) intended to tell us?

2) What is the immune response study (870.7800) intended to tell us.

3) What is the Acute injection toxicity/pathogeniaty (885.3200) intended to tell us.

4) What are the data requirements for a bacteriophog Are the above ones required now??

5) Did the company submit the above studies

E. coli EUP BPPD review 2/4/11
Tracy Lantz to: Eliot Harrison 03/09/2011 03:54 PM
Cc: Velma Noble, Dennis Edwards, Kelly Sherman

Dear Mr. Harrison,

Attached is a revised DER for the pending Phage EUP and Temporary Tolerance Exemption applications. Please discard the previous version of the DER dated 1/12/2011; it has been superseded by the attached revised DER (dated 2/4/11).

We revised the DER to remove any discussion of the following 5 human studies that were included within the data submission volume that was assigned MRID 47786803:

-- Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. (1998). Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15. (MRID 47786803, pages 86-95 of 231)
-- Bruttin, A. and Brussow, H. (2005). Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878. (MRID 47786803, pages 177-181 of 231)
-- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. and Wedgewood, R.J. (1975). Defective Antibody Response to Bacteriophage X174 in Down Syndrome. J. Pediatrics 86, 201-211. (MRID 47786803, pages 108-112 of 231)
-- Ochs, H.D., Buckley, R.H., Kobayashi, R.H, Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. and herchfeld, M.S. (1992). Antibody Responses to Bacteriophage X174 in Patients with Adenosine Deaminase Deficiency. Blood 80, 1163-1171. (MRID 47786803, pages 113-121 of 231)
-- Sulakvelidze, A., Alavidze, Z. and Morris Jr., J.G. (2001). Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659 (MRID 47786803, pages 96-106 of 231)

At this time, EPA will not be relying on these five human studies. If you would like the Agency to consider relying upon the articles by Alisky, Bruttin, and/or Sulakvelidze, you must comply with the requirements at 40 CFR 26.1303 by submitting documentation of the ethical conduct of these studies. For Alisky and Sulakvelidze, we would need 1303 data on all of the human studies reviewed in these review articles.

The Agency may not rely on the research discussed in Bruttin or Lopez in this action because these are intentional exposure human studies involving children, and reliance on such studies is prohibited by 40 CFR 26.1703 unless the data are crucial to a decision to impose a more stringent regulatory restriction that would improve public health (as provided at 40 CFR 26.1706). Since this is an application for a new EUP and temporary tolerance exemption, not a decision that would result in a more stringent regulatory outcome, the conditions required for the Agency to consider using Bruttin or Lopez under 40 CFR 26.1706 are not present.

Please let me know if you need any additional information on this matter.

Tracy Lantz

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

----- Forwarded by Tracy Lantz/DC/USEPA/US on 03/09/2011 03:47 PM -----

From:	cts/cts/QP/USEPA/US@EPA
To:	Tracy Lantz/DC/USEPA/US@EPA
Date:	03/03/2011 06:29 PM
Subject:	E. coli EUP BPPD review 2/4/11

Please open the attached document. This document was digitally sent to you

using an HP Digital Sending device. [Untitled].pdf

EUP/temp tolerance write up for Steve
Dennis Edwards to: Joan Harrigan-Farrelly 03/24/2011 12:15 PM
Cc: Velma Noble, Tracy Lantz

Joan,

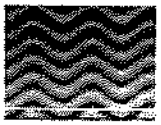
See if the brief description below is adequate for you to send to Steve regarding the temp. tolerance document. Otherwise I will add more.

Dennis

Intralytix, Inc. has submitted an experimental use permit application (EUP) for use of a new active ingredient *E.coli* 0157:H7 specific bacteriophage, which is a bacterial virus specific against *E.coli* 0157:H7. The product is a preparation of lytic bacteriophages highly specific for *E. coli* 0157:H7. When the bacteriophage encounters the *E.coli*, they sequentially attach to the bacterial cell surface, inject their DNA into the bacterium, replicate within the bacterial host, and liberate the phage progeny by lysing the bacterium, rendering it definitively and permanently incapable of causing subsequent food borne illness. Laboratory experiments under controlled conditions have been successful. The objective of the EUP is to evaluate the ability of this phage product to control *E. coli* 0157:H7 on both food and non-food contact surfaces in a real world situation, a field study. Trials will be conducted at facilities owned and operated by Tyson Fresh Meats, Inc. The product will only be used as an adjuvant with and prior to the application of registered food contact sanitizers.

Since the bacteriophage product will be applied to food contact surfaces such as food processing machinery and counter tops, a temporary tolerance is also required. Because this is the first food tolerance for this active ingredient, the temporary tolerance must be approved by the Office Director.

BPPD has evaluated the information submitted in support of the temporary tolerance. Specifically, the bacterial cultures used to produce the bacteriophage were screened to ensure that they do no produce toxins, pathogenicity factors or lysogenized phage. The company is using non-toxigenic and non-pathogenic bacteri free of bacteriophage for production of the pesticide which removes a lot of risk concerns. Thus, BPPD concerns are satisfied. OGC has reviewed the temporary tolerance write-up and concurred.



Re: Escherichia coli O157:H7 Specific Bacteriophages; Temporary Exemption From the Requirement of a Tolerance

Elizabeth Thomas to: Tracy Lantz 03/t6/20tt 04:47 PM

Cc: Dennis Edwards, Velma Noble, James-L Graves, John-A Richards, Melissa Chun, Angela Hofmann, Karen Angulo, Debbie-E Thomas

History: This message has been replied to.

Tracy:

Regarding the comment in your e-mail below concerning who is to sign this document...I have consulted with James Graves, OPP Team Leader in our office, and he in turn had a discussion with Angela Hofmann, Director of the Regulatory Coordination Staff (RCS). Both Angela Hofmann and James Graves state that this document should be signed by the Director for OPP and not your Division Director.

Angela Hofmann states the following regarding this issue in an e-mail to James Graves dated today, 3/16/11:

James -

As we discussed, the OPP OD's redelegation on record related to the authority to sign a tolerance or an exemption from a tolerance is dated July 22, t994. Although that document redelegates the "authority to establish, revise or revoke tolerances" in most cases, it also contains several specific limitations, including one for the establishment of the FIRST tolerance or an exemption from a tolerance for a chemical, which it reserves to the Office Director. My records do not indicate any change to that redelegation, but if the program has a memo documenting a change, please get a copy of our files.

Melissa Chun is my point person for delegations, so please copy her on any follow-up. Thanks.

- Angela

Angela Hofmann
Director of Regulatory Coordination for Chemical
Safety and Pollution Prevention (OCSPP)
U.S. Environmental Protection Agency (EPA)
Mailcode: 7101M
1200 Pennsylvania Ave., N.W., Washington, DC 20460
(Location: EPA East Building Room 3426 A)
Phone number: 202-564-0258; Fax number: 202-564-0263

Thus, based on Angela Hofmann's response above please provide any documentation your management has that states that your Division Director can sign your document ASAP.

Thanks,

Elizabeth Thomas

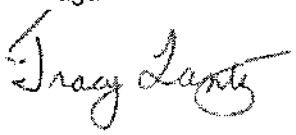
Tracy Lantz Thanks for your assistance on this document. Th... 03/16/2011 03:45:43 PM

From: Tracy Lantz/DC/USEPA/US
To: Elizabeth Thomas/DC/USEPA/US@EPA
Cc: Dennis Edwards/DC/USEPA/US@EPA, Velma Noble/DC/USEPA/US@EPA
Date: 03/t6/20t1 03:45 PM
Subject: Re: Escherichia coli O t57:H7 Specific Bacteriophages; Temporary Exemption From the Requirement of a Tolerance

Thanks for your assistance on this document.

The correct 40 CFR reference (listed on page 8) is 40 CFR 158.2140 (c)
I have checked with my management and they have indicated the signature block should indicate Joan Harrigan-Farrelly.
This is not signed by Steve Bradbury. These types of actions have been delegated down to the division directors.

Thanks again.



Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

Elizabeth Thomas Tracy: Since we keep missing each other via ph... 03/16/2011 12:53:48 PM

From: Elizabeth Thomas/DC/USEPA/US
To: Tracy Lantz/DC/USEPA/US@EPA
Date: 03/16/2011 12:53 PM
Subject: Escherichia coli O157:H7 Specific Bacteriophages; Temporary Exemption From the Requirement of a Tolerance

Tracy:

Since we keep missing each other via phone I am writing the questions/comments I have in the hope that you will be able to respond to this e-mail ASAP. Please understand that your document is on hold until I receive a response from you.

Here are the questions:

1. In Unit III., on page 8, the paragraph that begins with the words "Based on the published literature...." You make a reference to 40 CFR 158.690(c). This section does not exist per e-cfr (electronic code of federal regulations). Please let me know what the correct citation should be.

2. In Unit VIII, on page 13, I recommend removing a portion of the text you have listed here; the text that begins with "The Agency.....and ending with "food processing plants." You state this very same text already in Unit VI., on page 10 and 11, in the first paragraph of this document. Further, the template only asks you to fill out the following for Unit VIII.:

Therefore, a temporary exemption is established for residues of [insert biochemical/microbial/PIP-name on commodity].

Please advise.

Here are the comments:

1. In the DATES: section (page 1) and in Unit I.C. (page 4; top paragraph) of the document, you removed

the templated language regarding the calculation of the dates to read "[Insert date of posting]." This is incorrect. The correct language to use is this "[*insert date of publication in the* Federal Register]." This is the language that was in the template and it's the correct language to use. I will make this change. In the future leave this language as is.

2. In Unit IV.A.1., on page 9, you make references to "E. coli" several times. I am going to change this to read "Escherichia coli" to be consistent with the terminology.

3. On page 16, regarding the signature block, the person who is supposed to sign this document is the Director, Office of Pesticide Programs (Steven Bradbury). When you are adding a section the director for OPP signs the document; not the division director. I will make the change to the title.

Also, please do not type the name of the person who will sign the document. Our office types the name in when the document comes back signed to us.

Thanks,

Elizabeth Thomas

Sent for typesetting 3/19/12

BILLING CODE 6560–50–P

[redacted]

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 180

[redacted] **[EPA–HQ–OPP–20[10]–0274; FRL–XXXX–X]**

[redacted] **RIN 2070–[Tolerances are exempt, unless proposed by EPA. If proposed, use the proposed rule's RIN.]**

[*Escherichia coli* O157:H7 Specific Bacteriophages,.]; Temporary Exemption From the Requirement of a Tolerance

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This regulation establishes a temporary exemption from the requirement of a tolerance for residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria when applied/used [on food contact surfaces in food processing plants] in accordance with the terms of Experimental Use Permit (EUP) No. [74234-EUP-2]. Intralytix, Inc. submitted a petition to EPA under the Federal Food, Drug, and Cosmetic Act (FFDCA), requesting the temporary tolerance exemption. This regulation eliminates the need to establish a maximum permissible level for residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria. The temporary tolerance exemption expires on [April 1, 2013].

DATES: This regulation is effective [*Insert date of posting*]. Objections and requests for hearings must be received on or before [*Insert date 60 days after posting]* and must be

filed in accordance with the instructions provided in 40 CFR part 178 (see also Unit I.C. of the **SUPPLEMENTARY INFORMATION**).

ADDRESSES: EPA has established a docket for this action under docket identification (ID) number EPA–HQ–OPP–20[10]–0274. All documents in the docket are listed in the docket index available at *http://www.regulations.gov*. Although listed in the index, some information is not publicly available, e.g., Confidential Business Information (CBI) or other information whose disclosure is restricted by statute. Certain other material, such as copyrighted material, is not placed on the Internet and will be publicly available only in hard copy form. Publicly available docket materials are available in the electronic docket at *http://www.regulations.gov*, or, if only available in hard copy, at the OPP Regulatory Public Docket in Rm. S–4400, One Potomac Yard (South Bldg.), 2777 S. Crystal Dr., Arlington, VA. The Docket Facility is open from 8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays. The Docket Facility telephone number is (703) 305–5805.

FOR FURTHER INFORMATION CONTACT: Tracy Lantz, Antimicrobials Division (7510P), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001; telephone number: [703-308-6415]; e-mail address: *Lantz.tracy@epa.gov*.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

You may be potentially affected by this action if you are an agricultural producer, food manufacturer, or pesticide manufacturer. Potentially affected entities may include, but are not limited to:

- Crop production (NAICS code 111).
- Animal production (NAICS code 112).
- Food manufacturing (NAICS code 311).
- Pesticide manufacturing (NAICS code 32532).

This listing is not intended to be exhaustive, but rather provides a guide for readers regarding entities likely to be affected by this action. Other types of entities not listed in this unit could also be affected. The North American Industrial Classification System (NAICS) codes have been provided to assist you and others in determining whether this action might apply to certain entities. If you have any questions regarding the applicability of this action to a particular entity, consult the person listed under **FOR FURTHER INFORMATION CONTACT**.

B. How Can I Get Electronic Access to Other Related Information?

You may access a frequently updated electronic version of 40 CFR part 180 through the Government Printing Office's e-CFR site at *http://www.gpoaccess.gov/ecfr*. [If harmonized test guidelines are cited, insert the following: To access the harmonized test guidelines referenced in this document electronically, please go to *http://www.epa.gov/ocspp* and select "Test Methods and Guidelines."]

C. How Can I File an Objection or Hearing Request?

Under FFDCA section 408(g), 21 U.S.C. 346a, any person may file an objection to any aspect of this regulation and may also request a hearing on those objections. You

must file your objection or request a hearing on this regulation in accordance with the instructions provided in 40 CFR part 178. To ensure proper receipt by EPA, you must identify docket ID number EPA–HQ–OPP–20[10]–0274 in the subject line on the first page of your submission. All objections and requests for a hearing must be in writing, and must be received by the Hearing Clerk on or before [*insert date 60 days after posting*]. Addresses for mail and hand delivery of objections and hearing requests are provided in 40 CFR 178.25(b).

In addition to filing an objection or hearing request with the Hearing Clerk as described in 40 CFR part 178, please submit a copy of the filing that does not contain any CBI for inclusion in the public docket . Information not marked confidential pursuant to 40 CFR part 2 may be disclosed publicly by EPA without prior notice. Submit a copy of your non-CBI objection or hearing request, identified by docket ID number EPA–HQ–OPP–2010–0274, by one of the following methods:

• *Federal eRulemaking Portal*: *http://www.regulations.gov*. Follow the on-line instructions for submitting comments.

• *Mail*: Office of Pesticide Programs (OPP) Regulatory Public Docket (7502P), Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460–0001.

• *Delivery*: OPP Regulatory Public Docket (7502P), Environmental Protection Agency, Rm. S–4400, One Potomac Yard (South Bldg.), 2777 S. Crystal Dr., Arlington, VA. Deliveries are only accepted during the Docket Facility's normal hours of operation (8:30 a.m. to 4 p.m., Monday through Friday, excluding legal holidays). Special

arrangements should be made for deliveries of boxed information. The Docket Facility telephone number is (703) 305–5805.

II. Background and Statutory Findings

In the **Federal Register** of [May 5, 2010] ([Vol. 75, NO. 86] FR [24692]) (FRL–8820-7), EPA issued a notice pursuant to section 408(d)(3) of FFDCA, 21 U.S.C. 346a(d)(3), announcing the filing of a pesticide tolerance petition (PP[9G7585]) by Intralytix, Inc., 701 East Pratt Street, Baltimore, MD 21202. The petition requested that 40 CFR part 180 be amended by establishing a temporary exemption from the requirement of a tolerance for residues of *Escherichia coli* O157:H7 Bacteriophages. This notice referenced a summary of the petition prepared by the petitioner [Intralytix, Inc.] which is available in the docket, *http://www.regulations.gov*: There were no comments received in response to the notice of filing]

Section 408(c)(2)(A)(i) of FFDCA allows EPA to establish an exemption from the requirement for a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the exemption is "safe." Section 408(c)(2)(A)(ii) of FFDCA defines "safe " to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Pursuant to section 408(c)(2)(B) of FFDCA, in establishing or maintaining in effect an exemption from the requirement of a tolerance, EPA must take into account the factors set forth in section 408(b)(2)(C) of FFDCA, which require EPA to give special consideration to exposure of infants and children to the pesticide chemical

residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue...." Additionally, section 408(b)(2)(D) of FFDCA requires that the Agency consider "available information concerning the cumulative effects of a particular pesticide's residues" and "other substances that have a common mechanism of toxicity."

EPA performs a number of analyses to determine the risks from aggregate exposure to pesticide residues. First, EPA determines the toxicity of pesticides. Second, EPA examines exposure to the pesticide through food, drinking water, and through other exposures that occur as a result of pesticide use in residential settings.

III. Toxicological Profile

Consistent with section 408(b)(2)(D) of FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

Phages are naturally occurring viruses infecting bacteria. They are found in soil and water and in association with plants and animals, including humans. Bacteriophages are obligate parasites of bacteria, which means they attach to, infect, and reproduce in bacteria. Phages are host-specific for bacteria, with specific bacteriophages attacking only one bacterial species and most frequently only one strain within a bacterial species. As such, phages do not attack other beneficial bacteria. In addition, there is no evidence for bacteriophages infecting any other life form, including humans, except bacteria. Thus, non-target organisms, such as mammals, birds, fish, plants, and other wildlife, are

not affected by exposure to bacteriophages. Humans and other animals commonly consume bacteriophages as they are abundantly found in water, on plant surfaces and in foods such as ground beef, pork sausage, chicken, oysters, cheese, fresh mushrooms, and lettuce. In addition, phages are common commensals of the human gut and likely play an important role in regulating populations of various bacteria in the gastrointestinal tract. As cited in public literature, phages have been used for more than 80 years as therapeutic agents with no ill effects and are active against bacteria that cause many infections and human diseases.

Since bacteriophage do not infect humans, there is not a human health risk concern from the bacteriophages themselves. The potential concerns for human health risk from bacteriophages relate to their interaction with the bacteria they infect. If bacteriophage do not lyse (i.e., break open) the bacterial cell they infect, there is a possibility the cell will survive the infection and incorporate any DNA carried by the bacteriophage in its genome (i.e. lysogenize). If genes for shigatoxins I and II, often associated with pathogenic strains of *E. coli* O157:H7, are carried by a lysogenized bacteriophage into an atoxigenic *Escherichia coli,* there is a possibility, in theory, to convert a commensal and harmless bacterium into a pathogen. This theoretical risk is handled in three ways for this tolerance exemption: (1) Only lytic bacteriophage are used; (2) Bacteriophage covered by this tolerance exemption are DNA sequenced to ensure they do not have the ability to convey shigatoxins I and II; and (3) Host bacteria used to grow bacteriophage also are atoxigenic in that they do not carry DNA sequences capable of shigatoxin production.

To address the infectivity and toxicity endpoints for oral, pulmonary and injection exposures, the petitioner provided publicly available information documenting a lack of mammalian toxicity or infectivity associated with bacteriophages due to the specificity of bacteriophages attachment and attack to a narrow range of bacterial strains. As a result, the public literature demonstrates that phages pose little to no risk to humans even with the known wide exposure in food and the environment.

Based on the published literature and information submitted in accordance with the Tier I toxicology data requirements set forth in 40 CFR 158.690(c), the Tier II and Tier III toxicology data requirements also set forth therein were not triggered and, therefore, not required in connection with this action.

IV. Aggregate Exposures

In examining aggregate exposure, section 408 of FFDCA directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

A. Dietary Exposure

1. *Food.* [All phages, including those at issue in this action, are similar in nature in that they are host-specific, attacking only bacteria. Published literature submitted by the registrant, and other publically available literature, indicate that humans are exposed to phages daily, and these phages are commonly found in humans, having no known adverse effects. Indeed, humans and other animals routinely consume phages when they eat food such as raw produce and cheese. For example, it is reported that 1000 (10^3) to 5

x 10^5 phages can be isolated routinely per gram (g) of high quality cheese. Pathogenic microorganisms are often found in foods; therefore, it is not surprising that one study found *E. coli* and coliphages in 11 of 12 foods purchased at retail markets. In this study, 10 purchases of each of the 12 foods were made. All 10 of the fresh ground beef purchases were contaminated with *E. coli*, and all 10 contained coliphages. In addition to ground beef, *E. coli* and coliphages were found in chicken, fresh pork, fresh oyster, fresh mushrooms, lettuce, chicken pot pie, biscuit dough, deli loaf, deli roasted turkey, and package roasted chicken. Another example of phages in food has been *Propionibacterium freundenreichii* phage found in concentrations as high as 1.4 x 10^6/gm of swiss cheese.

The use of the bacteriophages covered by this tolerance in food processing plants on food contact surfaces could result in some residues of these bacteriophages on food. The Agency anticipates that food coming into contact with these surfaces could get residues of the phages on them and foods with E. coli O157:H7 may end up with more phages on them as the bacteriophages covered by this tolerance exemption infect the bacteria and produce progeny. 2. *Drinking water exposure.* The *Escherichia coli* bacteriophages covered by this tolerance exemption are not intended for use in drinking water, nor are the approved uses likely to result in these bacteriophages reaching surface water or ground water that might be used as drinking water. Use sites include food processing facilities.

B. Other Non-Occupational Exposure

[Since *Escherichia coli* bacteriophages subject to this tolerance exemption are only intended to be applied to food contact surfaces in food processing plants, the

potential for non-occupational, non-dietary exposures (i.e., dermal and inhalation exposures) to these phages by the general population, including infants and children, is highly unlikely.]

V. Cumulative Effects from Substances with a Common Mechanism of Toxicity

Section 408(b)(2)(D)(v) of FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information" concerning the cumulative effects of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

EPA has not found lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria to share a common mechanism of toxicity with any other substances. Moreover, bacteriophage that meet these conditions do not appear to produce a toxic metabolite produced by other substances. Therefore, for the purposes of this action, EPA has assumed that lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria do not have a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see EPA's website at *http://www.epa.gov/pesticides/cumulative.*

VI. Determination of Safety for U.S. Population, Infants and Children

[1. U.S. population. Based on the fact that bacteriophages are host-specific and do not cause harm to human health, except in theoretical instances that the Agency is avoiding through its conditions on this exemption, there is reasonable certainty that no

harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

2. Infants and children. FFDCA section 408 (b)(2)(C) provides that EPA shall apply an additional tenfold margin of exposure (MOE) for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data base on toxicity and exposure, unless EPA determines that a different MOE will be safe for children. MOEs, which are often referred to as uncertainty (safety) factors, are incorporated into EPA risk assessments either directly, or through the use of a MOE analysis or by using uncertainty factors in calculating a dose level that poses no appreciable risk. As previously mentioned in the toxicological profile, humans, including infants and children, have been exposed to phages generally through food and water, where they are commonly found, and through decades of therapeutic use, with no known or reported adverse effects. Based on all available information, the Agency concludes that lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria are non-toxic to mammals, including infants and children. Because there are no threshold effects of concern to infants, children, and adults when lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria are used as labeled, the Agency concludes that the additional

MOE is not necessary to protect infants and children and that not adding any additional MOE will be safe for infants and children.]

VII. Other Considerations

A. Analytical Enforcement Methodology

[An analytical method is not required for enforcement purposes since the Agency is establishing an exemption from the requirement of a tolerance without any numerical limitation.]

B. International Residue Limits

In making its tolerance decisions, EPA seeks to harmonize U.S. tolerances with international standards whenever possible, consistent with U.S. food safety standards and agricultural practices. EPA considers the international maximum residue limits (MRLs) established by the Codex Alimentarius Commission (Codex), as required by FFDCA section 408(b)(4). The Codex Alimentarius is a joint U.N. Food and Agriculture Organization/World Health Organization food standards program, and it is recognized as an international food safety standards-setting organization in trade agreements to which the United States is a party. EPA may establish a tolerance that is different from a Codex MRL; however, FFDCA section 408(b)(4) requires that EPA explain the reasons for departing from the Codex level.:

The Codex has not established a MRL for lytic bacteriophages that are specific to ***Escherichia coli* O157:H7**, sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria.

C. Revisions to Petitioned-For Tolerances

In its petition PP 9G7585, Intralytix requested that the Agency establish a tolerance exemption for residues of *Escherichia coli* O157:H7 specific bacteriophages. The Agency is narrowing the scope of the tolerance exemption to residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria because that is the category of bacteriophages for which the Agency can make a safety finding.

VIII. Conclusion

The Agency concludes that there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria, including all anticipated dietary exposures and all other exposures for which there is reliable information, when used according to label directions, as a microbial on food contact surfaces in food processing plants. Therefore, a temporary exemption is established for residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria.

IX. Statutory and Executive Order Reviews

This final rule establishes a tolerance under section 408(d) of FFDCA in response to a petition submitted to the Agency. The Office of Management and Budget (OMB) has exempted these types of actions from review under Executive Order 12866, entitled *Regulatory Planning and Review* (58 FR 51735, October 4, 1993). Because this final rule has been exempted from review under Executive Order 12866, this final rule is not subject to Executive Order 13211, entitled *Actions Concerning Regulations That*

Significantly Affect Energy Supply, Distribution, or Use (66 FR 28355, May 22, 2001) or Executive Order 13045, entitled *Protection of Children from Environmental Health Risks and Safety Risks* (62 FR 19885, April 23, 1997). This final rule does not contain any information collections subject to OMB approval under the Paperwork Reduction Act (PRA), 44 U.S.C. 3501 *et seq.*, nor does it require any special considerations under Executive Order 12898, entitled *Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations* (59 FR 7629, February 16, 1994).

Since tolerances and exemptions that are established on the basis of a petition under section 408(d) of FFDCA, such as the tolerance in this final rule, do not require the issuance of a proposed rule, the requirements of the Regulatory Flexibility Act (RFA) (5 U.S.C. 601 *et seq.*) do not apply.

[redacted]

This final rule directly regulates growers, food processors, food handlers, and food retailers, not States or tribes, nor does this action alter the relationships or distribution of power and responsibilities established by Congress in the preemption provisions of section 408(n)(4) of FFDCA. As such, the Agency has determined that this action will not have a substantial direct effect on States or tribal governments, on the relationship between the national government and the States or tribal governments, or on the distribution of power and responsibilities among the various levels of government or between the Federal Government and Indian tribes. Thus, the Agency has determined that Executive Order 13132, entitled *Federalism* (64 FR 43255, August 10, 1999) and Executive Order 13175, entitled *Consultation and Coordination with Indian Tribal Governments* (65 FR 67249, November 9, 2000) do not apply to this final rule. In

addition, this final rule does not impose any enforceable duty or contain any unfunded mandate as described under Title II of the Unfunded Mandates Reform Act of 1995 (UMRA) (Public Law 104–4).

This action does not involve any technical standards that would require Agency consideration of voluntary consensus standards pursuant to section 12(d) of the National Technology Transfer and Advancement Act of 1995 (NTTAA), Public Law 104–113, section 12(d) (15 U.S.C. 272 note).

X. Congressional Review Act

The Congressional Review Act, 5 U.S.C. 801 *et seq.*, generally provides that before a rule may take effect, the agency promulgating the rule must submit a rule report to each House of the Congress and to the Comptroller General of the United States. EPA will submit a report containing this rule and other required information to the U.S. Senate, the U.S. House of Representatives, and the Comptroller General of the United States prior to publication of this final rule in the **Federal Register**. This final rule is not a "major rule" as defined by 5 U.S.C. 804(2).

List of Subjects in 40 CFR Part 180

Environmental protection, Administrative practice and procedure, Agricultural commodities, Pesticides and pests, Reporting and recordkeeping requirements.

Dated: _____

_____

Joan Harrigan Farrelly
Director, Antimicrobials Division

Therefore, 40 CFR chapter I is amended as follows:

PART 180--[AMENDED]

1. The authority citation for part 180 continues to read as follows:

Authority: 21 U.S.C. 321(q), 346a and 371.

2. Section 180.- is added to subpart D to read as follows:

§180.- [***Escherichia coli* O157:H7 Specific Bacteriophages; temporary exemption from the requirement of a tolerance**].

[A temporary exemption from the requirement of a tolerance is established for residues of lytic bacteriophages that are specific to ***Escherichia coli* O157:H7,** sequence negative for shiga toxins I and II, and grown on atoxigenic host bacteria when used/applied on food contact surfaces in food processing plants in accordance with the terms of Experimental Use Permit (EUP) No. 74234-EUP-2. This temporary exemption expires on April 1, 2013.]

E. coli EUP BPPD review 2/4/11
Tracy Lantz to: Eliot Harrison 03/09/2011 03:54 PM
Cc: Velma Noble, Dennis Edwards, Kelly Sherman

Dear Mr. Harrison,

Attached is a revised DER for the pending Phage EUP and Temporary Tolerance Exemption applications. Please discard the previous version of the DER dated 1/12/2011; it has been superseded by the attached revised DER (dated 2/4/11).

We revised the DER to remove any discussion of the following 5 human studies that were included within the data submission volume that was assigned MRID 47786803:

-- Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. (1998). Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15. (MRID 47786803, pages 86-95 of 231)
-- Bruttin, A. and Brussow, H. (2005). Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878. (MRID 47786803, pages 177-181 of 231)
-- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. and Wedgewood, R.J. (1975). Defective Antibody Response to Bacteriophage X174 in Down Syndrome. J. Pediatrics 86, 201-211. (MRID 47786803, pages 108-112 of 231)
-- Ochs, H.D., Buckley, R.H., Kobayashi, R.H. Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. and herchfeld, M.S. (1992). Antibody Responses to Bacteriophage X174 in Patients with Adenosine Deaminase Deficiency. Blood 80, 1163-1171. (MRID 47786803, pages 113-121 of 231)
-- Sulakvelidze, A., Alavidze, Z. and Morris Jr., J.G. (2001). Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659 (MRID 47786803, pages 96-106 of 231)

At this time, EPA will not be relying on these five human studies. If you would like the Agency to consider relying upon the articles by Alisky, Bruttin, and/or Sulakvelidze, you must comply with the requirements at 40 CFR 26.1303 by submitting documentation of the ethical conduct of these studies. For Alisky and Sulakvelidze, we would need 1303 data on all of the human studies reviewed in these review articles.

The Agency may not rely on the research discussed in Bruttin or Lopez in this action because these are intentional exposure human studies involving children, and reliance on such studies is prohibited by 40 CFR 26.1703 unless the data are crucial to a decision to impose a more stringent regulatory restriction that would improve public health (as provided at 40 CFR 26.1706). Since this is an application for a new EUP and temporary tolerance exemption, not a decision that would result in a more stringent regulatory outcome, the conditions required for the Agency to consider using Bruttin or Lopez under 40 CFR 26.1706 are not present.

Please let me know if you need any additional information on this matter.

Tracy Lantz

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) [illegible]-8481

----- Forwarded by Tracy Lantz/DC/USEPA/US on 03/09/2011 03:47 PM -----

From:	[illegible]cts/QP/USEPA/US@EPA
To:	Tracy Lantz/DC/USEPA/US@EPA
Date:	[illegible]3/2011 06:29 PM
Subject:	[illegible]li EUP BPPD review 2/4/11

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5 Human Studies in submitted in support of Phage EUP and Temporary Tolerance Exemption

Kelly Sherman to: Tracy Lantz 02/14/2011 10:35 AM

Cc: Angela Huskey, Joel Gagliardi, Velma Noble, John Kough, Dennis Edwards, Laura Parsons

History: This message has been replied to.

Tracy,

As we discussed at the meeting on February 2nd, I reviewed the 5 human studies (public literature articles) that were submitted in support of the pending Phage EUP and Temporary Tolerance Exemption applications. At this time, EPA may not rely on any of these 5 studies in any regulatory actions under FIFRA or FFDCA. The details are provided in the attached memo, which I encourage you to keep in the file for this action. Three of the five studies may be usable in the future. If that is of interest to the applicant, they will need to submit the ethical conduct documentation per the requirements at 40 CFR 26.1303.

My understanding is that Joel Gagliardi has revised the DER to remove any discussion of these 5 articles. Since you provided an earlier version of the DER that discussed the 5 human studies to Eliot Harrison (the applicant's representative), what you should do is send the revised DER to Mr. Harrison along with an email explaining why the DER was revised. You don't need to send the ethics memo to Mr. Harrison - an email similar to the text below will be sufficient.

Please let me know if you have any questions

47786803 Five Bacteriophage Human Tox Studies.pdf

Kelly Sherman
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-8401

Dear Mr. Harrison,

Attached is a revised DER for the pending Phage EUP and Temporary Tolerance Exemption applications [or use whatever language is appropriate]. Please discard the previous version of the DER dated 1/12/2011; it has been superceded by the attached revised DER (dated xxxxxx).

We revised the DER to remove any discussion of the following 5 human studies that were included within the data submission volume that was assigned MRID 47786803:

-- Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. (1998). Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15. (MRID 47786803, pages 86-95 of 231)
-- Bruttin, A. and Brussow, H. (2005). Human Volunteers Reciving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878. (MRID 47786803, pages 177-181 of 231)
-- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. and Wedgewood, R.J. (1975). Defective

Antibody Response to Bacteriophage X174 in Down Syndrome. J. Pediatrics 86, 201-211. (MRID 47786803, pages 108-112 of 231)
-- Ochs, H.D., Buckley, R.H., Kobayashi, R.H, Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. and herchfeld, M.S. (1992). Antibody Responses to Bacteriophage X174 in Patients with Adenosine Deaminase Deficiency. Blood 80, 1163-1171. (MRID 47786803, pages 113-121 of 231)
-- Sulakvelidze, A., Alavidze, Z. and Morris Jr., J.G. (2001). Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659 (MRID 47786803, pages 96-106 of 231)

At this time, EPA will not be relying on these five human studies. If you would like the Agency to consider relying upon the articles by Alisky, Bruttin, and/or Sulakvelidze, you must comply with the requirements at 40 CFR 26.1303 by submitting documentation of the ethical conduct of these studies. For Alisky and Sulakvelidze, we would need 1303 data on all of the human studies reviewed in these review articles.

The Agency may not rely on the research discussed in Bruttin or Lopez in this action because these are intentional exposure human studies involving children, and reliance on such studies is prohibited by 40 CFR 26.1703 unless the data are crucial to a decision to impose a more stringent regulatory restriction that would improve public health (as provided at 40 CFR 26.1706). Since this is an application for a new EUP and temporary tolerance exemption, not a decision that would result in a more stringent regulatory outcome, the conditions required for the Agency to consider using Bruttin or Lopez under 40 CFR 26.1706 are not present.

Please let me know if you need any additional information on this matter.

----- Forwarded by Kelly Sherman/DC/USEPA/US on 02/16/2011 10:09 PM -----

From: Tracy Lantz/DC/USEPA/US
To: Kelly Sherman/DC/USEPA/US@EPA
Date: 02/02/2011 04:04 PM
Subject: Fw: EUP 74234-E BPPD review

This is the review which I forwarded to the consultant.
The other review which I mentioned was an efficacy review, no human studies issues there.

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

----- Forwarded by Tracy Lantz/DC/USEPA/US on 02/02/2011 04:02 PM -----

From: Tracy Lantz/DC/USEPA/US
To: "Eliot Harrison" <eharrison@lewisharrison.com>
Cc: Velma Noble/DC/USEPA/US@EPA, Dennis Edwards/DC/USEPA/US@EPA
Date: 02/24/2010 06:40 PM

Subject: Fw: EUP 74234-E BPPD review

I am forwarding two reviews to you. The first one is attached below. This is the review we received from BPPD. There appears to be some additional information needed which once submitted would be sent back to review.

I'm sending this to you as an e-mail instead of an official letter since I am concerned that I will not have a chance to compose a letter before I leave town for the Indoor Air Quality Association meeting. I feel it is important for you to have this review sooner rather than later.

Tracy Lantz

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

---- Forwarded by Tracy Lantz/DC/USEPA/US on 02/24/2010 06:25 PM -----

From: cts/cts/QP/USEPA/US
To: Tracy Lantz/DC/USEPA/US@EPA
Date: 02/24/2010 03:19 PM
Subject: EUP 74234-E BPPD review

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY &
POLLUTION PREVENTION

February 14, 2011

MEMORANDUM

SUBJECT: Ethics Screening for Five Human Studies Contained in MRID 47786803

TO: Tracy Lantz
Antimicrobials Division

FROM: Kelly Sherman
Human Research Ethics Reviewer
Office of the Director

REF: Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. (1998). Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15. (MRID 47786803, pages 86-95 of 231)

Bruttin, A. and Brussow, H. (2005). Human Volunteers Reciving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878. (MRID 47786803, pages 177-181 of 231)

Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. and Wedgewood, R.J. (1975). Defective Antibody Response to Bacteriophage ΦX174 in Down Syndrome. J. Pediatrics 86, 201-211. (MRID 47786803, pages 108-112 of 231)

Ochs, H.D., Buckley, R.H., Kobayashi, R.H, Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. and herchfeld, M.S. (1992). Antibody Responses to Bacteriophage ΦX174 in Patients with Adenosine Deaminase Deficiency. Blood 80, 1163-1171. (MRID 47786803, pages 113-121 of 231)

Sulakvelidze, A., Alavidze, Z. and Morris Jr., J.G. (2001). Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659 (MRID 47786803, pages 96-106 of 231)

I have screened the five articles referenced above, which report research with human subjects. These articles were submitted to EPA in June 2009 in support of an Experimental Use

Permit (EUP) and Temporary Tolerance Exemption Request for ECP-100™ containing three lytic monophages specific for *E. coli* 0157:H7. The articles were contained within a single data volume, which has been assigned MRID 47786803.

At this time, EPA will not be relying on any of these five studies in any regulatory decisions under FIFRA or FFDCA. Below is a discussion of the ethics considerations that apply to each of the studies.

1) Alisky 1998

Alisky (1998) is a literature review of 27 studies about the therapeutic use of bacteriophage. Because this article was submitted to EPA in 2009, after the effective date of EPA's Amended Rule for the Protection of Human Subjects of Research (April 7, 2006), it is subject to 40 CFR 26 Subpart M. The submission therefore should have included documentation of the ethical conduct of all of the underlying articles in the literature review that report research with human subjects (as those terms are defined at 40 CFR 26.1102(d) and (e)). Until and unless EPA receives the required ethics information on the human studies reviewed in this article, EPA will not proceed with an evaluation of whether to rely on this article in any regulatory actions under FIFRA or FFDCA.

2) Bruttin 2005

Bruttin (2005) reports research with human subjects involving intentional exposure, as those terms are defined at 40 CFR 26.1102. In the study, fifteen healthy adult volunteers received doses of *Escherichia* coli phage T4 and a placebo in drinking water over a four week period and were evaluated for toxic effects and bioavailability of the bacteriophage. Because this research was conducted for the purpose of identifying or measuring a toxic effect, review by the Human Studies Review Board would be required prior to a decision by EPA to rely on the data in a regulatory action under FIFRA or FFDCA (40 CFR 26.1602(b)(2)).

At this time, EPA does not plan to rely on this study in any regulatory actions under FIFRA or FFDCA. If that position changes in the future, the Agency will proceed with a thorough review of the science and ethics of this research, and will present this study for review by the Human Studies Review Board.

3) Lopez 1975

EPA reliance on Lopez (1975) in actions under FIFRA or FFDCA is prohibited by 40 CFR 26.1703 because some of the subjects were children under age 18.

The provision at 40 CFR 26.1706 which allows EPA to rely on research that is not acceptable under the standards in sections 26.1703 through 26.1705 is not applicable here because the data have been submitted to support an EUP and new temporary tolerance exemption. In order for section 26.1706 to be applicable, the data must be a crucial piece of information supporting a more stringent regulatory restriction that would improve the protection of public health.

4) Ochs 1992

EPA reliance on Ochs (1992) in actions under FIFRA or FFDCA is prohibited by 40 CFR 26.1703 because all of the subjects were children under age 18.

The provision at 40 CFR 26.1706 which allows EPA to rely on research that is not acceptable under the standards in sections 26.1703 through 26.1705 is not applicable here because the data have been submitted to support an EUP and new temporary tolerance exemption. In order for section 26.1706 to be applicable, the data must be a crucial piece of information supporting a more stringent regulatory restriction that would improve the protection of public health.

5) Sulakvelidze 2001

Sulakvelidze (2001) is a literature review of 18 studies about the therapeutic use of bacteriophage. Because this article was submitted to EPA in 2009, after the effective date of EPA's Amended Rule for the Protection of Human Subjects of Research (April 7, 2006), it is subject to 40 CFR 26 Subpart M. The submission therefore should have included documentation of the ethical conduct of all of the underlying articles in the literature review that report research with human subjects (as those terms are defined at 40 CFR 26.1102(d) and (e)). Until and unless EPA receives the required ethics information on the human studies reviewed in this article, EPA will not proceed with an evaluation of whether to rely on this article in any regulatory actions under FIFRA or FFDCA.

218

asked Angela to send most recent draft to Joel

Intralytix E. coli O157:H7 specific bacteriophage
Joel Gagliardi to: Tracy Lantz 02/08/2011 11:50 AM
Cc: John Kough, Angela Huskey

Here are the revised data waiver requests and food tolerance exemption petition reviews:

Intralytix_O157H7_Phage_DERs_REV-1.docx

All of the sections below are directly applicable to the food tolerance exemption petition and the entire review, or the most relevant parts, should replace the current references given in what we intend to publish. We should not mention the vaccine work since it is a dated reference.

The crux of the risk assessment food / safety finding is that bacteriophage only infect bacteria, their host range is limited as shown by host range testing, these bacteriophage are shown to be lytic and not prone to lysogeny which could increase their chance for carrying host DNA, the host bacteria used for cultivation are atoxigenic, and bacteriophage are generally already present in multiple foods, waters and the environment at similar levels proposed for uses here.

If someone sends me the current document (mine is a month old) being sent for comments I can make edits directly.
Joel

Presence in the Environment:

According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5 \times 10^8$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained $10^3\text{-}10^5$ PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

- Ashelford, K.E., Day, M.J. and Fry, J.C. 2003. Elevated Abundance of Bacteriophage Infecting Bacteria in Soil. Appl. Environ. Microbiol. 69, 285-289.
- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
- Boehme, J., Frisher, M.E., Jiang, S.C., Kellogg, C.A., Pichard, S., Rose, J.B., Steinway, C. and Paul, J.H. 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Ma. Ecol. Prog. Ser. 97, 1–10.
- Børsheim, K.Y. 1993. Native marine bacteriophage. FEMS Microbiol. Ecol. 102, 141–159.

- Calci, K.R., Burkhardt III, W., Watkins, W.D. & Rippey, S.R. 1998. Occurrence of Male-Specific Bacteriophage in Feral and Domestic Animal Wastes, Human Feces, and Human Associated Wastewaters. Appl. Environ. Microbiol. 64, 5027-5029.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. 92, 77–87.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
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- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127. - Maranger, R. & Bird, D.E. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. 31, 141–151.
- Noble, R.T. & Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.
- Proctor, L.M. & Fuhrman, J.A. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60–62.
- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.
- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:
According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature

review stating "Bacteriophage are commonly consumed by humans via various foods. In this context, bacteriophage have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophage. For example, bacteriophage were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from $3.3\text{-}4.4\text{x}10^{10}$ PFU/100 g of fresh chicken, up to $3.5\text{x}10^{10}$ PFU/100 g of fresh pork, and up to $2.7\text{x}10^{10}$ PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of $10^{1}\text{-}10^{5}$ PFU/100 g meats and up to 10^{5} PFU/g (10^{7} PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/

- Armon, R., Araujo, R., Kott, Y., Lucena, F. and Jofre, J. 1997. Bacteriophage of enteric bacteria in drinking water, comparison of their distribution in two countries. J. Appl. Microbiol. 83, 627-633.
- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. 2003. Isolation and Characterization of *Campylobacter* Bacteriophage from Retail Poultry. Appl. Environ. Microbiol. 69, 4511-4518.
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Health Effects:
Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects.

Transduction, Lysogeny and Bacteriophage Sequencing:
The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis of bacteriophage was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:
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Host Range Testing:
Data showing that monophage do not lyse 5 strains of *Listeria monocytogenes* , 5 species of *Salmonella* (*enteritidis* , *typhimurium* , *newport* , *paratyphi* B, *dublin*), 5 strains of *Staphylococcus aureus* or 5 strains of *Pseudomonas aeruginosa* was submitted in MRID 481521-02. Also included was testing of individual monophage for lysis against non-O157:H7 *E. coli* strains. ECML-4 and ECML-117 each lysed 1 of 76 tested strains, while ECML-134 lysed 18 strains including the one lysed by EMCL-117. ECML-134 is grown on *E. coli* Ec211/ ECOR-56 / ATCC 35375 which is reported as type O6:H1 while ECML-4 and ECML-117 are grown on type O7157:H7 *E. coli* strains. In total these phage lyse non-O157:H7 *E. coli* strains in less than 9% of tested cases.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

FEB 04 2011

MEMORANDUM

***** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION *****

SUBJECT: Data waiver requests and Temporary Food Tolerance Exemption for ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.

TO: Tracy Lantz
Regulatory Management Branch I
Antimicrobials Division (7510-P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511-P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511-P)

ACTION REQUESTED: Updated literature review to support data waiver requests and the food tolerance exemption petition for lytic monophages specific for *E. coli* O157:H7.

CONCLUSION: Waiver requests for: Acute Oral Toxicity / Pathogenicity; Acute Pulmonary Toxicity / Pathogenicity; Acute Injection Toxicity / Pathogenicity; Cell Culture; Acute Oral Toxicity; Acute Dermal Toxicity; Acute Inhalation Toxicity; Acute Eye Irritation; and Acute Dermal Irritation: **ACCEPTABLE**. Temporary Food Tolerance Exemption: **ACCEPTABLE**.

DATA REVIEW RECORD:

Active Ingredient:	Lytic monophages specific for *E. coli* O157:H7.
Product Name:	ECP-100™.
Company Name:	Intralytix, Inc.
EPA Reg. No.:	74234-EUP-E.
Chemical Number:	016432.
Decision Number:	416027.
DP Barcode:	380630.
MRID Nos.:	477868-03; 481521-01; 481521-02.

Background:
New guidance for using data generated from testing in humans necessitated a re-analysis of the literature supporting waiver requests and the food tolerance exemption petition.

REVIEW SUMMARY:
Study Type: Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).
MRID Nos.: 477868-03; upgraded by 481521-01 and 481521-02.
Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study Summary: Bacteriophage are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese consumed without any known harmful effects. Bacteriophage are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product. Analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic and the other two are already reported as atoxigenic in the literature. Bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification: ACCEPTABLE.

Study Type: Temporary Food Tolerance Exemption.
Test Material: Lytic monophages for *Escherichia coli* O157:H7.
Study Summary: Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated

drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese, without any known harmful effects after consumption. Bacteriophage are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product. Peer- reviewed literature or analysis of host strain and bacteriophage properties show the host strains are atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes in the bacteriophage, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification: ACCEPTABLE.

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).
MRID Nos.	477868-03; upgraded by 481521-01 and 481521-02.
Test Material	ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study No.	ECP-100/ SA001.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Waiver Requests for Microbial Pesticide Toxicology Data Requirements and Discussion of Safety Issues; Test for the Presence of Shiga Toxin Genes Stx-1 and Stx-2 in Ec211, or ATCC 35375; Lytic Activity of Component Monophages.
Author	Eliot Harrison; Chandi D. Carter; Alexander Sulakvedlidze, Ph.D.
Study Completed	May 30, 2009; May 19, 2010; June 1, 2010.
Study Summary	Bacteriophage are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese consumed without any known harmful effects. Bacteriophage are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product. Analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic and the other two are already reported as atoxigenic in the literature. Bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Signed and dated GLP statements were provided; These studies were either not subject to the requirements of 40 CFR Part 160, the requirements were not met, or the submitter does not know if GLP was followed for data collection.

to address toxicology of compounds and

The registrant included a thorough literature review and set of rationale to waive requirements for toxicology, pathogenicity, infectivity and irritation testing for the component monophage. In addition, MSDS for inert ingredients, and their status as minimal risk were submitted by email. Since this is both a manufacturing-use and end-use product without registered TGAIs, there is only one set of data waivers submitted.

RATIONALE:
Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English work, and in a search of Western/English language literature for any reported adverse effects, in a few cases reporting controlled scientific studies. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment, including in non-polluted and treated drinking water, and in foods and feeds, without any known harmful effects.

Inert Ingredients: [redacted]

Presence in the Environment:
According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5 \times 10^8$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained 10^3-10^5 PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

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- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
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- Børsheim, K.Y. 1993. Native marine bacteriophage. FEMS Microbiol. Ecol. 102, 141–159.
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- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127.
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- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.
- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophage are commonly consumed by humans via various foods. In this context, bacteriophage have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the

ground beef and chicken meat sold at retail contain various levels of various bacteriophage. For example, bacteriophage were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from 3.3-4.4x10^{10} PFU/100 g of fresh chicken, up to 3.5x10^{10} PFU/100 g of fresh pork, and up to 2.7x10^{10} PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of 10^1-10^5 PFU/100 g meats and up to 10^5 PFU/g (10^7 PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/
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Health Effects:

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Transduction, Lysogeny and Bacteriophage Sequencing:
The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis of bacteriophage was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:

- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Host Range Testing:
Data showing that monophage do not lyse 5 strains of *Listeria monocytogenes*, 5 species of *Salmonella* (*enteritidis*, *typhimurium*, *newport*, *paratyphi* B, *dublin*), 5 strains of *Staphylococcus aureus* or 5 strains of *Pseudomonas aeruginosa* was submitted in MRID 481521-02. Also included was testing of individual monophage for lysis against non-O157:H7 *E. coli* strains. ECML-4 and ECML-117 each lysed 1 of 76 tested strains, while ECML-134 lysed 18 strains including the one lysed by EMCL-117. ECML-134 is grown on *E. coli* Ec211/ ECOR-56 / ATCC 35375 which is reported as type O6:H1 while ECML-4 and ECML-117 are grown on type O7157:H7 *E. coli* strains. In total these phage lyse non-O157:H7 *E. coli* strains in less than 9% of tested cases.

Deficiencies: None.

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Temporary Food Tolerance Exemption Petition.
MRID Nos.	None.
Test Material	Lytic monophages for *Escherichia coli* O157:H7.
Study No.	None given.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Petition Requesting a Temporary Tolerance Exemption from the Requirement of a Tolerance for *E. coli* O157:H7 Specific Bacteriophage used on Food-Contact Surfaces in Food Processing Plants.
Author	None given.
Study Completed	None given.
Study Summary	Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese, without any known harmful effects after consumption. Bacteriophage are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product. Peer- reviewed literature or analysis of host strain and bacteriophage properties show the host strains are atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes in the bacteriophage, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^{9} PFU/mL working solution applied to food and non-food contact surfaces. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria, Salmonella, Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Not applicable.

Presence in the Environment:

According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^{9} per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996,

Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5\text{x}10^{8}$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained 10^{3}-10^{5} PFU/100 mL sewage with an approximate decrease of 10^{1} PFU/100 mL with treatment (Calci et al. 1998).

References:

- Ashelford, K.E., Day, M.J. and Fry, J.C. 2003. Elevated Abundance of Bacteriophage Infecting Bacteria in Soil. Appl. Environ. Microbiol. 69, 285-289.
- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
- Boehme, J., Frisher, M.E., Jiang, S.C., Kellogg, C.A., Pichard, S., Rose, J.B., Steinway, C. and Paul, J.H. 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Ma. Ecol. Prog. Ser. 97, 1–10.
- Børsheim, K.Y. 1993. Native marine bacteriophage. FEMS Microbiol. Ecol. 102, 141–159.
- Calci, K.R., Burkhardt III, W., Watkins, W.D. & Rippey, S.R. 1998. Occurrence of Male-Specific Bacteriophage in Feral and Domestic Animal Wastes, Human Feces, and Human Associated Wastewaters. Appl. Environ. Microbiol. 64, 5027-5029.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. 92, 77–87.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
- Fuhrman, J.A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399, 541-548.
- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127.
- Maranger, R. & Bird, D.E. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. 31, 141–151.
- Noble, R.T. & Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.
- Proctor, L.M. & Fuhrman, J.A. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60–62.
- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.

- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophage are commonly consumed by humans via various foods. In this context, bacteriophage have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophage. For example, bacteriophage were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from 3.3-4.4x10^{10} PFU/100 g of fresh chicken, up to 3.5x10^{10} PFU/100 g of fresh pork, and up to 2.7x10^{10} PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of 10^{1}-10^{5} PFU/100 g meats and up to 10^{5} PFU/g (10^{7} PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/
- Armon, R., Araujo, R., Kott, Y., Lucena, F. and Jofre, J. 1997. Bacteriophage of enteric bacteria in drinking water, comparison of their distribution in two countries. J. Appl. Microbiol. 83, 627-633.
- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. 2003. Isolation and Characterization of *Campylobacter* Bacteriophage from Retail Poultry. Appl. Environ. Microbiol. 69, 4511-4518.
- Gautier, M., Rouault, A., Sommer, P. and Briandet, R. 1995. Occurrence of *Propionibacterium freudenreichii* Bacteriophage in Swiss Cheese. Appl. Environ. Microbiol. 61, 2572-2576.
- Greer, G.G. 2005. Bacteriophage Control of Foodborne Bacteria. J. Food Prot. 68, 1102-1111.
- Kennedy Jr., J.E., Oblinger, J.L. and Bitton, G. 1984. Recovery of Coliphages from

Chicken, Pork Sausage and Delicatessen Meats. J. Food Prot. 47, 623-626.
- Kennedy Jr., J.E., Wei, C.I. and Oblinger, J.L. 1986. Methodology for Enumeration of Coliphages in Foods. Appl. Environ. Microbiol. 51, 956-962.
- Maciorowski, K.G., Pillai, S.D. and Ricke, S.C. 2001. Presence of Bacteriophage in Animal feed as Indicators of fecal Contamination. J. Environ. Sci. Health B36, 699-708.
- Whitman, P.A. and Marshall, R.T. 1971. Isolation of Psychrophilic Bacteriophage-Host Systems from Refrigerated Food Products. Appl. Microbiol. 22, 220-223.

Health Effects:
Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects.

Transduction, Lysogeny and Bacteriophage Sequencing:
The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis of bacteriophage was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:
- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Host Range Testing:
Data showing that monophage do not lyse 5 strains of *Listeria monocytogenes*, 5 species of *Salmonella* (*enteritidis*, *typhimurium*, *newport*, *paratyphi* B, *dublin*), 5 strains of *Staphylococcus aureus* or 5 strains of *Pseudomonas aeruginosa* was submitted in MRID 481521-02. Also included was testing of individual monophage for lysis against non-O157:H7 *E. coli* strains. ECML-4 and ECML-117 each lysed 1 of 76 tested strains, while ECML-134 lysed 18 strains including the one lysed by EMCL-117. ECML-134 is grown on *E. coli* Ec211/ ECOR-56 / ATCC 35375 which is reported as type O6:H1 while ECML-4 and ECML-117 are grown on type O7157:H7 *E. coli* strains. In total these phage lyse non-O157:H7 *E. coli* strains in less than 9% of tested cases.

Deficiencies: None.

Pages 67-90 Privileged attorney-client communication

RE: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

Eliot Harrison to: Tracy Lantz 11/15/2010 06:37 PM

Hi Tracy,
Here's the previously submitted label with the expiration date. I noticed that the Storage & Disposal language wasn't updated. Do you want to include that in a cover letter or do you want me to update.
Regards,
Eliot

-----Original Message-----
From: Lantz.Tracy@epamail.epa.gov [mailto:Lantz.Tracy@epamail.epa.gov]
Sent: Monday, November 15, 2010 6:12 PM
To: Eliot Harrison
Subject: RE: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

Please send your current label. In addition, the BPPD review has indicated that the label must include a use by date which is within 60 days of manufacture.

Thanks
[Embedded image moved to file: pic00608.jpg)

rec'd 11/15

Eliot will update S+D.

FOR EXPERIMENTAL-USE ONLY

ECP-100

For the control of *E. coli* 0157:H7 on Food and Non-Food Contact Surfaces in Food Processing Plants

NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT OR COOPERATOR OF THE EPA-APPROVED EXPERIMENTAL USE PROGRAM

Active Ingredient

E. coli 0157:H7 specific Bacteriophages* 0.00027%

Inert Ingredients.....99.99973%

*Comprised of the following monophages: ECML-4, ECML-117 and ECML-134. Nominal titer of ECP-100 is 10^{10} PFU/ml

KEEP OUT OF REACH OF CHILDREN

CAUTION

EPA Experimental Use Permit No. 74234-EUP-

EPA Establishment Number:

Net Contents:

Intralytix Inc.
701 East Pratt St.
Baltimore, MD 21202

Expiration Date: (60 days from the date of manufacture will be inserted)

this seems to agree with what Joel reviewed

ask Joel if this is OK- I did not receive a package w/ label he reviewed.

Directions for Use

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

ECP-100 can is for use on food and non-food-contact surfaces in food-processing plants. Prior to application, add 1 part of ECP-100 into a clean container. Then add 9 parts of non-chlorinated water. If water is taken from a chlorinated source, allow the water to sit at room temperature for 24 hours prior to addition to ECP-100. After dilution, the use-solution or working titer of ECP-100 is approximately 10^9 PFU/ml. Apply the ECP-100 use-solution by either spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to ECP-100 application.

Only use ECP-100 as an adjunct to EPA registered food-contact surface sanitizers. Apply ECP-100 at least 5 minutes prior to using an EPA registered sanitizer following the use-instructions for the EPA registered sanitizer.

Precautionary Statements

Hazards to Humans: Avoid contact with eyes, skin or clothing. Wash thoroughly with soap and water after handling.

Storage and Disposal

Do not contaminate water, food or feed by storage or disposal.

Storage: Store in original plastic container at 4°C.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on-site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or dispose of in a sanitary landfill, or by incineration, of if allowed by state and local authorities, by burning. If burned, stay out of smoke.

FOR EXPERIMENTAL-USE ONLY
ECP-100

For the control of *E. coli* 0157:H7 on Food and Non-Food Contact Surfaces in Food Processing Plants

NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT OR COOPERATOR OF THE EPA-APPROVED EXPERIMENTAL USE PROGRAM

Lytic ✓

Active Ingredient
E. coli 0157:H7 specific Bacteriophages* 0.00027%

Inert Ingredients.....99.99973%

*Comprised of the following monophages: ECML-4, ECML-117 and ECML-134. Nominal titer of ECP-100 is 10^{9} PFU/ml

not needed don't have to be

KEEP OUT OF REACH OF CHILDREN

this specific

CAUTION

EPA Experimental Use Permit No. 74234-EUP-
EPA Establishment Number:

Net Contents:

Intralytix Inc.
701 East Pratt St.
Baltimore, MD 21202

per Joel + Dennis 1/6/11

Directions for Use
It is a violation of federal law to use this product in a manner inconsistent with its labeling.

ECP-100 can is for use on food and non-food-contact surfaces in food-processing plants. Prior to application, add 1 part of ECP-100 into a clean container. Then add 9 parts of non-chlorinated water. If water is taken from a chlorinated source, allow the water to sit at room temperature for 24 hours prior to addition to ECP-100. After dilution, the use-solution or working titer of ECP-100 is approximately 10^{9} PFU/ml. Apply the ECP-100 use-solution by either spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to ECP-100 application.

Only use ECP-100 as an adjunct to EPA registered food-contact surface sanitizers. Apply ECP-100 at least 5 minutes prior to using an EPA registered sanitizer following the use-instructions for the EPA registered sanitizer.

Precautionary Statements
Hazards to Humans: Avoid contact with eyes, skin or clothing. Wash thoroughly with soap and water after handling.

Storage and Disposal
Do not contaminate water, food or feed by storage or disposal.
Storage: Store in original plastic container at 4°C.
Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on-site or at an approved waste disposal facility.
Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or dispose of in a sanitary landfill, or by incineration, of if allowed by state and local authorities, by burning. If burned, stay out of smoke.

Joel okd this label 8/2010

Email Notification of Negotiated Due Date
Pesticide Registration Improvement Act to: lantz.tracy 01/07/2011 08:22 AM
Sent by: DCOPPAPPS01
Please respond to Pesticide Registration

SUBJECT: Notification -- Due Date for Decision #416027 has been re-negotiated

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Please note: The PRIA Due Date for the following decision has been negotiated:

Decision Number:	416027
Original Due Date:	04/13/2010
Negotiated Due Date:	04/10/2011

You are a Reviewer assigned to a data package for this decision

---

This is an automatically generated notification message. Please do not reply to this address.

74234-EUP-E

202 564
2892

Conference Call: 1/6/11

w/ Angela Huskey 2:15 - 3:00
Joel Gagliardi
Dennis Edwards
Tracy Lantz

(I need to print her draft w/ comments (Angela 12/28/10))

Angela - how specific do we need to be? phages change + adapt over time

Joel - don't list nonphages on label or tolerance petition,
will continue to have to sequence each new phage

Angela - how to limit so they always need same criteria?
specify criteria in tolerance
"the lytic b. phage sequence negative for shigatox 1 + 2"

Dennis - modify Ingre statement

Joel - ~~[illegible]~~
phage only infect bacteria, E. coli normally in intestine
this phage is specific for E. coli bacteria
we ~~eat~~ eat phages that will ~~digest~~ digest E. coli phages
no none negative effects of phages
causes disease since it grows in stored ~~[illegible]~~ foods
toxins can build up in kidneys (very remote chance)
ingesting is common w/o effect.

conditions must be
Lytic
No toxins
Sequence - for Shigatoxin 1 + 2 and intimin ~~[illegible]~~ eae gene

95

human studies were public literature data.
need to make sure ~~we~~ we can side
human data w/o issue
Any time OPP is relying on human studies w/ have
to go thru for ethics approval.
Joel relied on reasonably current human studies
Angela w/ check w/ human studies folks
Dennis – our reg notice w/ specify where they ~~can~~ can
use the ~~product~~ product

Angela – waiving data ~~requirements~~ requirements – they may have
already applied by submitting public literature

Joel – waives are weight of evidence not to submit ~~studies~~ studies
Angela – we had public literature in place of studies
most data is ~~product~~ product chemistry data
Joel – we require they sent every public literature document
consistent w/ ~~their request~~ they request

Angela – ~~we~~ wants to discuss how p.l. supports each
of the data requirements

She wants to add sentence = "all phages w/ these ~~characteristics~~
characteristics don't cause any adverse effect."
Phages used – not adverse effects.

Dennis – we need to ask company to revise their tolerance?

a proposal w/ our same limits + add this to the docket
We won't put in table in 40CFR

Angela - we can prob explain what we are
doing differently based on what they submitted

Joel will write a topic sentence
Angela will reply to us next week.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Recommendation of Division Directors
Negotiated Due Dates

Decision#: D 416027	**Registration#: 74234-EUP-E**	**Petition #: 9G7585**
Fee Category: A520	**PRIA Decision Time Frame: 9 months**	
Submitted by: Velma Noble/Tracy Lantz	**Branch: RMBI**	**Date: 1/5/11**
Company: Intralytix, Inc		
Original Due Date: January 10, 2011	**Proposed New Due Date: April 10, 2011**	
Previous Negotiated Due Dates: two		
Is the "Fix" in-house? N/A	**If not, date "Fix" expected:**	

Issue (describe in detail): Agency review (conducted by BPPD) identified a number of concerns related to the submitted efficacy data, product characterization and temporary food tolerance exemption. The most critical issue to be addressed was whether this bacteriophage produces toxins, pathogenicity factors or lysogenized phage. Submission was renegotiated to allow company to address the deficiencies. Additional information was provided to the Agency but did not arrive by promised date of 6/14/10. Information arrived 7/16/10 thus the need for a second renegotiation. BPPD has completed the review of this additional data and indicated that all data requirements have now been satisfied. The Final Rule (FR) for the temporary tolerance exemption was drafted and sent to OGC for review and concurrence in December. OGC has raised a number of questions on the draft temporary tolerance exemption. These questions have prompted a meeting between OGC, AD, and BPPD on 1/6/11. Additional time is needed to address any additional concerns which may be raised by OGC, revise the temporary tolerance exemption, receive concurrence from OGC and issue the FR.

Summary of Deficiency Type(s): Not Submitted (N) Deficiencies (D)
Product Chemistry: ___ **Acute Tox:** ___ **Efficacy:** ____ **Labeling:** ____ **Other (describe):** ____

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates): The company was contacted in mid December regarding the need for additional time for OGC to complete the review of the temporary tolerance exemption and AD to revise accordingly. The consultant replied on 12/20/10 with a 90 day renegotiation.

"75 Day" Letter sent? _____ **(Date sent)** __X__ **No and reason for none?**

Concerns were discussed via phone call.

Rationale for Proposed Due Date:

New due date allows 90 additional days to finalize draft document and establish the temporary tolerance exemption.

Registrant notified that this is the last negotiation? ____ **Yes** ___X_ **Not Applicable**

Approve: ✓ 1-6-11 | **Disapprove:**

If disapproved, action to be taken:

CONCURRENCES

SYMBOL	OD or DOD Signature:	7510P	7510P	7510P			Date:		
SURNAME		L Lantz	Edwards	Harrigan Family					
DATE		1/5/11	1/5/11	1/5/11					

ECP-100, File Symbol No. 74234-EUP; PRIA Extension
Eliot Harrison
to:
Tracy Lantz
12/20/2010 06:11 PM
Show Details

Hi Tracy,
On behalf of Intralytix Inc., I am requesting that the PRIA deadline for experimental use permit (EUP)/temporary tolerance for ECP-100 be extended from January 10, 2011 until April 10, 2011. The reason for the extension is to allow the Agency additional time to process the temporary tolerance action.

If you have any questions about this request, please contact me at (202) 393-3903, ext. 14 or e-mail at eharrison@lewisharrison.com

Best regards,
Eliot

Read!! ✓ setup meeting ✓

Re: E coli bacteriophage temp. tol. exemption

Joel Gagliardi to: Angela Huskey 12/28/2010 02:24 PM

Cc: John Kough, Tracy Lantz

discuss → 1/6/11

202 564 2892 S-8771

Angela,

I can answer most of these questions directly;

1. How broadly do we intend to set this tolerance exemption? And relatedly, how broadly does the science support setting this tolerance exemption? With my very limited understanding of how these phages work, I see three different ways of setting this up (and which one we choose will direct the types of changes that will need to be made to the tolerance document):

Phage infect only bacteria. The host range is known only for those hosts tested for lysis (by plaque formation).

(a) The tolerance itself says "E. coli O157:H7 specific bacteriophages". This is what the applicants asked for, but it seems pretty broad. It would appear to cover any bacteriophage that targets E. coli O157:H7. (If we do want it to be so broad and the science supports this, I would suggest different wording that would be clearer as to what we mean.) Given that we mention the specific monophages in the tolerance document and that we specifically tested these to ensure that they were safe, it seems that such a broad tolerance might cover some phages that might not be safe.

I do not mind the tolerance exemption being broad, though they are currently testing only three bacteriophage for an EUP so this is only a temporary tolerance. I would not limit the tolerance to any specific pathogenicity factors since other may be implicated in E. coli O157:H7 later - I am fine keeping the risk assessment with the reviews for this product.

(b) The science review refers to "lytic monophages specific for E. coli O157:H7", which seems narrower than the title of the tolerance exemption. Is it? If so, is it necessary to limit what is covered by the exemption to the "lytic monophages...." to avoid any possible safety concern? Also, we mention a few times in the science review that the phages cannot produce shigatoxins, and completely lyse or have no activity against other strains. Do we need to add these as criteria to our tolerance exemption, i.e., you won't qualify for the exemption unless you meet these criteria? Or does the term "E. coli O157:H7 specific bacteriophages" mean that there won't be any phages that meet that description that would not also meet the criteria?

We avoided safety concerns mainly through sequences of the phage and determination that the host bacteria did not contain shigatoxins. There is no test for lysogeny, just for lysis. Therefore, in testing where individual phage did not lyse tested E. coli, we cannot say for sure they did not lysogenize. Most lysed the target cells so we have very reasonable certainly, along with the sequencing and toxin checking, for food-use safety.

(c) The tolerance document refers to the three specific monophages that comprise the mixture: ECML-4, ECML-117, and ECML-134. Do we know if the mixture of bacteriophages is anticipated to change? Should we be specific about these monophages as the particular substances that are being exempted from a tolerance?

For this EUP it will not change, though they may want to test other phage later - and these, plus the host bacteria used to grow them in culture, would need a similar review, but perhaps a new tolerance exemption review can be avoided by keeping it nonspecific for now.

2. We refer to a few human studies in the science review. Were these actual studies submitted or were they just part of a literature review? Have they been submitted to our human studies review team to see if it is alright for the Agency to rely on these studies?

There are upwards of 10^{10} CFU bacteria per gram feces, and phage number perhaps 10x that level. They are very numerous and inseparable from bacterial populations. There are no standardized tests for toxicity/pathogenicity and no need to conduct them since phage can only infect bacteria. The literature cited was not for these phage rather for those tested for similar exposure patterns, in some cases for pharmaceutical uses. Phage are on the very safe side of risk assessments generally.

3. Is there some confusion about how the EUP will be conducted? The review notes that although the experiment is taking place at Tyson Fresh Meats plants, there is a reference to Perdue personnel in the documentation.

Yes, there is some confusion.

4. It is not really correct to say that we are waiving the data requirements for data where we obtain information relevant to those effects through public literature. Waivers are appropriate in instances where the particular data would be unhelpful or inappropriate based on the particular characteristics of the pesticide. When we obtain information related to the pesticide's effects (e.g., toxicity) through public literature, the data requirement is actually fulfilled.
There is no tox/path data for these particular phage, rather we relied on the weight of evidence approach to grant waivers. We could have just said 'not required' though this may not be standard practice for AD.
5. We say that there are a number of reports of studies done using bacteriophages as antibiotics or for other therapeutic reasons, and there have been no adverse effects reported as a result of such usage. This information is only helpful if it is relevant to the bacteriophages at issue here. How does that information apply to the bacteriophages at issue here? Are they the same types? Do all bacteriophages have the same characteristics? (I thought there were some that could be harmful.) We probably just need a sentence or two that explains why the historical use that has not revealed any adverse effects is relevant to the particular bacteriophages that we're concerned about in this exemption.
Bacteriophage are very numerous and exposure to them ubiquitous. The sole risk is gene transfer from pathogenic bacteria so we attempted to rule this out using non-pathogen hosts and sequencing the phage specific to this tolerance exemption. I am confident this is enough. The literature review mainly cites their relative ubiquity and safety for various exposures, except of course for the nagging rare ones that can transfer toxins.
6. On a related note, we say often that phages are found everywhere without any reported adverse effects, but can we really be so general? As I mentioned in #5, I thought some phages could be harmful. Shouldn't we be more careful about what general statements we make?
Phage are EVERYWHERE - and none are harmful. They may, however, transfer pathogenicity traits and/or toxin production genes between bacteria. We would not want this to occur in a food processing plant so we have them select primarily lytic phage (lysogenic phage insert into the host genome) and that lack pathogenicity factors, in this case shigatoxin genes. Other phage would have differing concerns. For example if this were phage active against Vibrio we would want to ensure that they could not carry Cholera toxin genes. There are a few dozen other examples. Since these are used to mitigate potential human pathogens we are being extra cautious and requiring sequencing of the specific phage.

****

Joel V. Gagliardi, Ph.D.
U.S. Environmental Protection Agency, Mailcode 7511-P
OCSPP, OPP, BPPD, Microbial Pesticides Branch
1200 Pennsylvania Avenue, NW
Washington, DC 20460

703-308-0116 - phone / 703-305-0118 or 703-308-7026 - fax
http://www.epa.gov/pesticides/biopesticides
****

Angela Huskey Attorney-Client Communication Attorney Work P... 12/28/2010 01:34:49 PM

From:	Angela Huskey/DC/USEPA/US
To:	Tracy Lantz/DC/USEPA/US@EPA
Cc:	John Kough/DC/USEPA/US@EPA, Joel Gagliardi/DC/USEPA/US@EPA
Date:	12/28/2010 01:34 PM
Subject:	E coli bacteriophage temp. tol. exemption

Attorney-Client Communication
Attorney Work Product
Pre-Decisional/Deliberative
Privileged and Confidential--Do Not Release

Tracy,

[redacted]

[redacted]

[redacted]

Thanks,
Angela

[attachment "EUP draft template temp tol Interlytix Ec O157 121610 JLK.TKL.ah.doc" deleted by Joel Gagliardi/DC/USEPA/US]

****

Angela M.D. Huskey
Office of General Counsel, Pesticides and Toxic Substances Law Office
Mail Code 2333A
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, D.C. 20460

Room 7426HH - Ariel Rios North
Phone: (202) 564-2892
Fax: (202) 564-5644

12/20/10 assigned to Angela Huskey @ OGC

Re: Temporary tolerance for a bateriophage
Tracy Lantz to: Chris Kaczmarek 12/16/2010 12:29 PM
Cc: Jonathan Fleuchaus, Dennis Edwards, Velma Noble

12/20/10 Eliot will renegotiate for 90 days

Hi, Chris and Jonathan,

Attached below is the draft temporary tolerance I have been working on for E. coli bacteriophages.
I used the Westlaw example which was provided (below) and worked closely with John Kough in BPPD.
I wrote this based on the PRIA due data of 1/10/11. I am aware that we will need to renegotiate in order to address your comments.
I will revise the dates in this document once I have a renegotiation in hand from the company.

EUP draft template temp tol Interlytix Ec 0157 121610 JLK.TKL.doc

Here is the memorandum/assessment from BPPD for this experimental use permit

Intralytix_0157H7_Phage_OERs.doc

Please provide your comments by Jan. 6, 2011.
Thanks in advance.

Tracy Lantz

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

Chris Kaczmarek Confidential Attorney-Client Communication 09/07/2010 12:21:09 PM

From:	Chris Kaczmarek/DC/USEPA/US
To:	Tracy Lantz/DC/USEPA/US@EPA
Cc:	Jonathan Fleuchaus/DC/USEPA/US@EPA
Date:	09/07/2010 12:21 PM
Subject:	Re: Temporary tolerance for a bateriophage

Confidential
Attorney-Client Communication
Attorney Work Product
Pre-Decisional/Deliberative -- Do Not Release Under FOIA Without Further Review

[redacted]

[redacted]

[redacted]m

[redacted].

Thanks, Chris!

Tracy Lantz	Hi Chris, My name is Tracy, I'm in AD and am cu...	09/07/2010 10:45:33 AM

Re: Fw: Temporary tolerance for a bacteriophage
John Kough to: Tracy Lantz 12/16/2010 07:10 AM
Cc: Dennis Edwards, Velma Noble

Tracy,

This draft looks good. I would suggest that you choose option #1 for the cumulative mechanism of toxicity. There is no indication that there is any mammalian toxicity for these bacteriophage so there cannot be a common mechanism of toxicity. And for the international residue limits, I am not aware of any international standards for these agents so again I would choose option #1: no international residue limits.

You have shown some great flexibility in doing this tolerance for an active that is somewhat outside the norm for AD. Thanks for asking the questions, putting up with the less than satisfactory explanations and getting the job done.

John K.

Tracy Lantz Thanks again for your assistance with this. Atta... 12/15/2010 07:55:20 PM

From: Tracy Lantz/DC/USEPA/US
To: John Kough/DC/USEPA/US@EPA
Cc: Dennis Edwards/DC/USEPA/US@EPA, Velma Noble/DC/USEPA/US@EPA
Date: 12/15/2010 07:55 PM
Subject: Re: Fw: Temporary tolerance for a bacteriophage

Thanks again for your assistance with this. Attached below is my latest draft.
I have a few questions at this point:

1) In the Cumulative Effects from Substances with a Common Mechanism of Toxicity section, should I use the boilerplate: "Option 1" or should I write some alternative language?

2) Which option should I select for International Residue Limits?

[attachment "EUP draft template temp tol Interlytix Ec O157 121510 JLK.TKL.doc" deleted by John Kough/DC/USEPA/US]

Just in case you need it, here is the example document I received from OGC.

[attachment "Westlaw_Document_11_11_10.pdf" deleted by John Kough/DC/USEPA/US]

Tracy Lantz Hi Chris, My name is Tracy, I'm in AD and am cu... 09/07/2010 10:45:33 AM

John Kough Tracy, Here is a version of the temporary toleran... 12/03/2010 02:20:28 PM
Tracy Lantz Here is the document I have been working on. P... 11/30/2010 05:38:18 PM

Tracy Lantz	Hi Chris, My name is Tracy, I'm in AD and am cu...	09/07/2010 10:45:33 AM

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Tracy Lantz to: John Kough 12/15/2010 07:55 PM
Cc: Dennis Edwards, Velma Noble

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2) Which option should I select for International Residue Limits?

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To: Tracy Lantz/DC/USEPA/US@EPA, Dennis Edwards/DC/USEPA/US@EPA
Date: 12/03/2010 02:20 PM
Subject: Re: Fw: Temporary toterance for a bacteriophage

Tracy,

Here is a version of the temporary tolerance I wrote for you. I have only addressed the product information and toxicity data requirements and stopped at1he aggregate exposure. I used the info in the memo you sent and the literature that was cited. Hope this gets you where you need. Let me know if you would like more help.

John K.

EUP draft template temp tol Interlytix Ec 0157 120310 JLK.doc

Tracy Lantz Here is the document I have been working on. P... 11/30/2010 05:38:18 PM

From:	Tracy Lantz/DC/USEPA/US
To:	John Kough/DC/USEPA/US@EPA
Date:	11/30/2010 05:38 PM
Subject:	Fw: Temporary tolerance for a bacteriophage

Here is the document I have been working on. Please let me know if there are sections that you think I should revise. At this point I have worked up to the end of page seven. This is where I got stuck.

[attachment "EUP draft template 112210.doc" deleted by John Kough/DC/USEPA/US]

Attached below is the example document that I received from Chris Kaczmarek.
I have been using this as a guide when writing my temporary tolerance.

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

----- Forwarded by Tracy Lantz/DC/USEPA/US on t1/30/2010 05:28 PM -----

From:	Chris Kaczmarek/DC/USEPA/US
To:	Tracy Lantz/DC/USEPA/US@EPA
Cc:	Jonathan Fleuchaus/DC/USEPA/US@EPA
Date:	09/07/2010 t2:21 PM
Subject:	Re: Temporary tolerance for a bateriophage

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[redacted]

Re: Fw: Temporary tolerance for a bacteriophage
John Kough to: Tracy Lantz, Dennis Edwards 12/03/2010 02:20 PM

Tracy,

Here is a version of the temporary tolerance I wrote for you. I have only addressed the product information and toxicity data requirements and stopped at the aggregate exposure. I used the info in the memo you sent and the literature that was cited. Hope this gets you where you need. Let me know if you would like more help.

John K.

looks good need to work on aggregate assess. to end.

EUP draft template temp tol Interlytix Eco 0157 120310 JLK.doc
Tracy Lantz Here is the document I have been working on. P... 11/30/2010 05:38:18 PM

From: Tracy Lantz/DC/USEPA/US
To: John Kough/DC/USEPA/US@EPA
Date: 11/30/2010 05:38 PM
Subject: Fw: Temporary tolerance for a bacteriophage

revised dated 12/15 — forwarded to John + Dennis w/ a few questions of 12/15/10

Here is the document I have been working on. Please let me know if there are sections that you think I should revise. At this point I have worked up to the end of page seven. This is where I got stuck.

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From: Chris Kaczmarek/DC/USEPA/US
To: Tracy Lantz/DC/USEPA/US@EPA
Cc: Jonathan Fleuchaus/DC/USEPA/US@EPA
Date: 09/07/2010 12:21 PM

Privileged attorney-client communication

Subject: Re: Temporary tolerance for a bateriophage

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Attorney-Client Communication
Attorney Work Product
Pre-Decisional/Deliberative -- Do Not Release Under FOIA Without Further Review

[redacted]

Thanks, Chris!

Tracy Lantz Hi Chris, My name is Tracy, I'm in AD and am cu... 09/07/2010 10:45:33 AM

11/30/10

How to address Hypersensitivity (discussion w/ John Kough)

insert E. coli ~~in~~ this canned section from OGC

Discuss: Literature citation

Immune Response

specific to Bacteriage shigatoxin pg 13

Pathogenicity Factors

Acute ~~injection~~ tox | path

summarize this info for the entire section

- lack of immune response from Joel here
- use immune system info p. 5
- all info about citing literature
- lack of pathog. + toxicity
- No toxins produced

* Literature also provides evidence that phages are not ineffective agents in humans based on used as therapeutic agents vectors in vaccines to control disease w/o adverse effects "

highlighted in ~~orange~~ orange

highlighted in yellow pg. 5

~~info from second review~~

13

p.g. 8 sequence analysis

EUP for ECP-100 74234-EUP-E

Notes:

Bacteriophages are viruses that only infect specific bacteria.

We (BPPD) accepted waiver requests for:

acute oral / patho.

acute pulmonary / patho.

acute injection / path. cell culture

acute oral

acute dermal

acute inhalation

acute eye irritation

acute dermal irritation

Test material: ECP-100 containing lytic monophages specific for E. Coli O157:H7

Accepted temporary food tolerance - bacteriophage host strains must be confirmed shigatoxin free

Bacteriophages currently reported in foods + feeds (meat, cheese) w/o harmful effects.

Issues:

Joel says a revised label + CSF were submitted which addressed deficiencies.

Where are they?

Need revised label with use by date w/in 60 days of manufacture

Is Eliot supposed to send current label. - I asked for use by date +100, do I have a docket?? is it the same docket which contains the notice of filing? (yes)

2010-0274

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 2 6 2010

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

***** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION *****

SUBJECT: Experiment Use Permit and Temporary Food Tolerance Exemption for ECP-100™ containing three lytic monophages specific for *E. coli* O157:H7.

TO: Tracy Lantz
Regulatory Management Branch I
Antimicrobials Division (7510-P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511-P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and Pollution Prevention Division (7511-P)

ACTION REQUESTED: Review deficiency responses for an EUP application with a temporary food tolerance exemption petition for lytic monophages specific for *E. coli* O157:H7.

CONCLUSION: Product identity and composition: **ACCEPTABLE** – a revised label must include a use-by date within 60 days from manufacture. Waiver requests for: Acute Oral Toxicity / Pathogenicity; Acute Pulmonary Toxicity / Pathogenicity; Acute Injection Toxicity / Pathogenicity; Cell Culture; Acute Oral Toxicity; Acute Dermal Toxicity; Acute Inhalation Toxicity; Acute Eye Irritation; and Acute Dermal Irritation: **ACCEPTABLE**. Temporary Food Tolerance Exemption: **ACCEPTABLE**. For registration, all bacteriophage host strains used for production must be confirmed shigatoxin free in a manner similar to analyses used herein for *E. coli* Ec211/ ECOR-56 / ATCC 35375.

DATA REVIEW RECORD:

Active Ingredient:	Lytic monophages specific for *E. coli* O157:H7.
Product Name:	ECP-100™.
Company Name:	Intralytix, Inc.
EPA Reg. No.:	74234-EUP-E.
Chemical Number:	016432.
Decision Number:	416027.
DP Barcode:	380630.
MRID Nos.:	477868-03; 481521-01; 481521-02.

Background:
The registrant submitted responses to Agency questions about use of ECP-100 during the EUP; the phage will be used as a pre-treatment adjunct to use of existing EPA registered food-contact surface sanitizers. The EUP sites will be supervised directly by Intralytix personnel and will initially be performed at a single Tyson Fresh Meats, Inc. plant in Dakota City, NE over 150,000 square feet using 1,800 gallons of ECP-100. An additional 7 sites in NE, WA, TX, KA (2 sites) IA and IL are planned over two years if favorable results are achieved at the initial test site. The registrant notes that all additional sites will be Tyson Fresh Meats, Inc. plants with Dean Danilson, Ph.D. as cooperator though they also refer to "Perdue personnel" as performing some testing, as listed in an undated Experimental Program for ECP-100 submitted by Intralytix, Inc.

PREVIOUS REVIEW SUMMARY:
Study Type: Product Identity (OPPTS 885.1100)
Manufacturing Process (OPPTS 885.1200)
Discussion of Formation of Unintentional Ingredients (OPPTS 885.1300)
Analysis of Samples (OPPTS 885.1400)
Certification of Limits (OPPTS 8850.1500)
Enforcement Analytical Method (OPPTS 830.1800)
Physical and Chemical Characteristics (OPPTS 830.6302-830.7300).
MRID Nos.: 477868-01; 477868-02.
Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study Summary: Three monophage active against a wide range of *E. coli* O157:H7 strains are produced in *E. coli* hosts which lyse as the phage matures ([redacted]

Classification: SUPPLEMENTAL but Upgradeable - *For the EUP:* Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; a revised CSF should be provided listing endotoxin as a contaminant, nominal concentrations of all inert ingredients, minimum PFU/ml for each monophage in ECP-100™ (consistent with the manufacturing process), removing the word 'specific' from the active ingredient description or provide supporting tests for review; a label matching the revised CSF should be provided with clear usage and dilution rates listed. ***For registration;*** Methodology (including reagents and protocols) for the PFGE, RFLP and amplicon identity tests should be submitted, information on toxicology for Gram-negative endotoxin, or product toxicology testing, to support the proposed endotoxin limit should be submitted and proposed endotoxin limits should be standardized in the manufacturing process and on the CSF; storage stability must be addressed by data and accompanied by a 'use-by' date on the label; an additional two batch analyses should be submitted.

Study Type: Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).
MRID Nos.: 477868-03.

Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study Summary: Bacteriophages are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^{9}$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^{3}-10^{5} PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP.
Classification: SUPPLEMENTAL but Upgradeable - ***For the EUP:*** Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided.

Study Type: Temporary Food Tolerance Exemption.
Test Material: Lytic monophages for *Escherichia coli* O157:H7; ECML-4, ECML-117 and ECML-134.
Study Summary: Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^{9}$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^{3}-10^{5} PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese without any known harmful effects.

Bacteriophages are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and peer- reviewed literature or analysis of host strain and bacteriophage properties show the host strains are atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes in the bacteriophage, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces.
Classification: SUPPLEMENTAL but Upgradeable - *For the EUP:* Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided; a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register.

CURRENT REVIEW SUMMARY:
Study Type: Product Identity (OPPTS 885.1100)
Manufacturing Process (OPPTS 885.1200)
Discussion of Formation of Unintentional Ingredients (OPPTS 885.1300)
Analysis of Samples (OPPTS 885.1400)
Certification of Limits (OPPTS 8850.1500)
Enforcement Analytical Method (OPPTS 830.1800)
Physical and Chemical Characteristics (OPPTS 830.6302-830.7300).
MRID Nos.: 481521-01.
Test **Material:** *E. coli* Ec211/ ECOR-56 / ATCC 35375.
Study Summary: PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted. A revised label and CSF were provided that addressed previous deficiencies. Storage stability data was not submitted.
Classification: ACCEPTABLE – a revised label must include a use-by date within 60 days from manufacture.

Study Type: Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).
MRID Nos.: 477868-03; upgraded by 481521-01 and 481521-02.
Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study Summary: Bacteriophages are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet

Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification: ACCEPTABLE.

Study Type: Temporary Food Tolerance Exemption.
Test Material: Lytic monophages for *Escherichia coli* O157:H7; ECML-4, ECML-117 and ECML-134.
Study Summary: Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and peer- reviewed literature or analysis of host strain and bacteriophage properties show the host strains are atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes in the bacteriophage,

which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria, Salmonella, Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.

Classification: ACCEPTABLE.

*** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION ***

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.

EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Product Identity (OPPTS 885.1100); Manufacturing Process (OPPTS 885.1200); Discussion of Formation of Unintentional Ingredients (OPPTS 885.1300); Analysis of Samples (OPPTS 885.1400); Certification of Limits (OPPTS 885.1500); Enforcement Analytical Method (OPPTS 830.1800); Physical and Chemical Characteristics (OPPTS 830.6302-830.7300).
MRID Nos.	481521-01.
Test Material	*E. coli* Ec211/ ECOR-56 / ATCC 35375.
Study No.	None given.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Titles of Reports	Test for the Presence of Shiga Toxin Genes Stx-1 and Stx-2 in Ec211, or ATCC 35375.
Author	Chandi D. Carter.
Study Completed	May 19, 2010.
Study Summary	PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted. A revised label and CSF were provided that addressed previous deficiencies. Storage stability data was not submitted.
Classification	**ACCEPTABLE** – a revised label must include a use-by date within 60 days from manufacture.
Good Laboratory Practice	A signed and dated (June 1, 2010) GLP statement was provided; This study was not conducted in accordance with requirements of 40 CFR Part 160.

I. MANUFACTURING PROCESS:

Table 1. Phage and host characteristics for manufacturing:

Phage	Host *E. coli* [1]	Other ID	Host type	Toxigenic	MOI [2]	Host OD_{600}	Phage OD_{600}
ECML-4	Ec149	87-23	O157:H7	No	0.001	0.2	0.05-0.15
ECML-117					0.01	0.2	0.7-0.8
ECML-134	Ec211 [3]	ECOR-56 / ATCC 35375	O6:H1	No [3]	0.001	0.2	0.01-0.05

[1] Free of endogenous phage by no lysis from cell-free supernatant to lawn of cells; [2] Multiplicity of infection as PFU/CFU;
[3] Multiplex PCR showed that genes for shigatoxins Stx-1 and Stx-2 were not present.

1) Multiplex PCR: Two separate published methods utilizing different forward and reverse PCR primers for Stx-1 and Stx-2 detection were utilized. Each assay was performed in triplicate from *E. coli* grown on L-broth as published by Paton and Paton 1998 and Belanger et al. 2002. Neither assay showed the expected bands for Stx-1 (180 or 185 bp) or Stx-2 (255 or 160 bp).

References:

- Belanger, S.D., M. Boissinot, C. Menard, F.J. Picard and M.G. Bergeron. 2002. Rapid Detection of Shiga Toxin-Producing Bacteria in Feces by Multiplex PCR with Molecular Beacon on the Smart Cycler. Journal of Clinical Microbiology 40(4):1436-1440.
- Paton, A.W. and J.C. Paton. 1998. Detection and Characterization of Shiga Toxigenic Escherichia coli by Using Multiplex PCR Assays for stx_1, stx_2, *eaeA*, Enterohemorrhagic *E. coli hylA*, rfb_{O111}, and rfb_{O157}. Journal of Clinical Microbiology 36(2):598-602.

Deficiencies: None.

Inert ingredient information may be entitled to confidential treatment

II. CERTIFICATION OF LIMITS: Table 5 lists the nominal concentration and certified limits for the ingredients in ECP-100™.

TABLE 5. Nominal CSF concentrations and certified limits for ECP-100™ a

Ingredients (CAS number)	PC Code	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
ECP-100 is a mixture of three (3) lytic monophages specific for *E. coli* O157:H7. The specific monopahges are: • ECML-4 [minimum 10^{10} PFU/mL]. • ECML-117 [minimum 10^{10} PFU/mL]. • ECML-134 [minimum 10^{10} PFU/mL].	016432	TGAI	0.00027	0.00024	0.00030
Inert Ingredients					
[redacted]					
Contaminants					
[redacted]					

[a]Data from CSF (7/14/2010) and MSDS (email from Eliot Harrison 12/1/2009).

Deficiencies: None.

VII. PHYSICAL AND CHEMICAL CHARACTERISTICS:

1) Storage Stability – the label advises to store the product at 4°C in the original container, otherwise this requirement is not addressed by data for maintenance of monophage or prevention of any contaminants growth.

Deficiencies: For Registration - Storage stability must be addressed by data; For the EUP - a 'use-by' date within 60 days of manufacture must be added to the label.

DATA EVALUATION RECORD

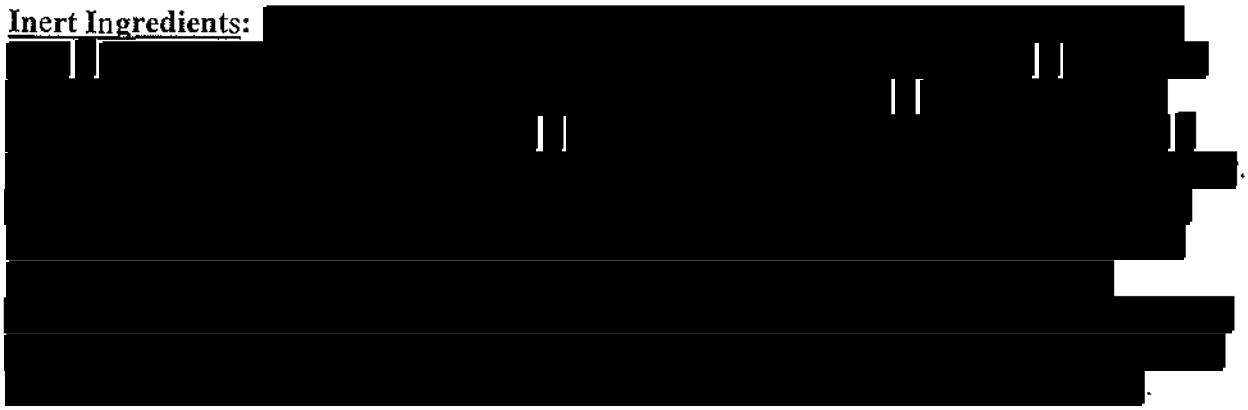
EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).
MRID Nos.	477868-03; upgraded by 481521-01 and 481521-02.
Test Material	ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study No.	ECP-100/ SA001.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Waiver Requests for Microbial Pesticide Toxicology Data Requirements and Discussion of Safety Issues; Test for the Presence of Shiga Toxin Genes Stx-1 and Stx-2 in Ec211, or ATCC 35375; Lytic Activity of Component Monophages.
Author	Eliot Harrison; Chandi D. Carter; Alexander Sulakvedlidze, Ph.D.
Study Completed	May 30, 2009; May 19, 2010; June 1, 2010.
Study Summary	Bacteriophages are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^{9}$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^{3}-10^{5} PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis of bacteriophage was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Signed and dated GLP statements were provided; These studies were either not subject to the requirements of 40 CFR Part 160, the requirements were not met, or the submitter does not know if GLP was followed for data collection.

The registrant included a thorough literature review and set of rationale to waive requirements for toxicology, pathogenicity, infectivity and irritation testing for the component monophage. In addition, MSDS for inert ingredients, and their status as minimal risk were submitted by email. Since this is both a manufacturing-use and end-use product without registered TGAIs, there is only one set of data waivers submitted.

RATIONALE:
Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English work, and in a search of Western/English language literature for any reported adverse effects, in a few cases reporting controlled scientific studies. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment, including in non-polluted and treated drinking water, and in foods and feeds, without any known harmful effects.

Inert Ingredients:



Presence in the Environment:
According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5 \times 10^8$ PFU/g

soil (Ashelford et al. 2003). Sewage plant effluents contained 10^3-10^5 PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

- Ashelford, K.E., Day, M.J. and Fry, J.C. 2003. Elevated Abundance of Bacteriophage Infecting Bacteria in Soil. Appl. Environ. Microbiol. 69, 285-289.
- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
- Boehme, J., Frisher, M.E., Jiang, S.C., Kellogg, C.A., Pichard, S., Rose, J.B., Steinway, C. and Paul, J.H. 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Ma. Ecol. Prog. Ser. 97, 1–10.
- Børsheim, K.Y. 1993. Native marine bacteriophages. FEMS Microbiol. Ecol. 102, 141–159.
- Calci, K.R., Burkhardt III, W., Watkins, W.D. & Rippey, S.R. 1998. Occurrence of Male-Specific Bacteriophage in Feral and Domestic Animal Wastes, Human Feces, and Human Associated Wastewaters. Appl. Environ. Microbiol. 64, 5027-5029.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. 92, 77–87.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
- Fuhrman, J.A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399, 541-548.
- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127.
- Maranger, R. & Bird, D.E. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. 31, 141–151.
- Noble, R.T. & Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.
- Proctor, L.M. & Fuhrman, J.A. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60–62.
- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.
- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors… healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophages are commonly consumed by humans via various foods. In this context, bacteriophages have been commonly isolated from a wide range of food products, including ground

beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophages. For example, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from $3.3\text{-}4.4\text{x}10^{10}$ PFU/100 g of fresh chicken, up to $3.5\text{x}10^{10}$ PFU/100 g of fresh pork, and up to $2.7\text{x}10^{10}$ PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of $10^1\text{-}10^5$ PFU/100 g meats and up to 10^5 PFU/g (10^7 PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/
- Armon, R., Araujo, R., Kott, Y., Lucena, F. and Jofre, J. 1997. Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. J. Appl. Microbiol. 83, 627-633.
- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. 2003. Isolation and Characterization of *Campylobacter* Bacteriophages from Retail Poultry. Appl. Environ. Microbiol. 69, 4511-4518.
- Gautier, M., Rouault, A., Sommer, P. and Briandet, R. 1995. Occurrence of *Propionibacterium freudenreichii* Bacteriophages in Swiss Cheese. Appl. Environ. Microbiol. 61, 2572-2576.
- Greer, G.G. 2005. Bacteriophage Control of Foodborne Bacteria. J. Food Prot. 68, 1102-1111.
- Kennedy Jr., J.E., Oblinger, J.L. and Bitton, G. 1984. Recovery of Coliphages from Chicken, Pork Sausage and Delicatessen Meats. J. Food Prot. 47, 623-626.
- Kennedy Jr., J.E., Wei, C.I. and Oblinger, J.L. 1986. Methodology for Enumeration of Coliphages in Foods. Appl. Environ. Microbiol. 51, 956-962.
- Maciorowski, K.G., Pillai, S.D. and Ricke, S.C. 2001. Presence of Bacteriophages in Animal feed as Indicators of fecal Contamination. J. Enciron. Sci. Health B36, 699-708.
- Whitman, P.A. and Marshall, R.T. 1971. Isolation of Psychrophilic Bacteriophage-Host Systems from Refrigerated Food Products. Appl. Microbiol. 22, 220-223.

Health Effects:

Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language

formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects (Alisky et al 1998, Sulakvelidze et al 2001). Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from the bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight (Lopez 1975, Ochs et al. 1992). *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication (Bruttin and Brussow 2005).

References:

-Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. 1998. Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15.
- Bruttin, A. & Brussow, H. 2005. Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878.
- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. & Wedgewood, R.J. 1975. Defective antibody response to bacteriophage ΦX174 in Down syndrome. J. Pediatrics 86, 207-211.
- Ochs, H.D., Buckley, R.H., Kobayashi, R.H., Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. & Herchfeld, M.S. 1992. Antibody Responses to Bacteriophage ΦX174 in Patients With Adenosine Deaminase Deficiency. Blood 80, 1163-1171.
-Sulakvelidze, A., Alavidze, Z. & Morris Jr., J.G. 2001. Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659.

Transduction, Lysogeny and Bacteriophage Sequencing:

The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis of bacteriophage was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:

- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Host Range Testing:

Data showing that monophage do not lyse 5 strains of *Listeria monocytogenes*, 5 species of *Salmonella* (*enteritidis*, *typhimurium*, *newport*, *paratyphi* B, *dublin*), 5 strains of *Staphylococcus aureus* or 5 strains of *Pseudomonas aeruginosa* was submitted in MRID 481521-02. Also

included was testing of individual monophage for lysis against non-O157:H7 *E. coli* strains. ECML-4 and ECML-117 each lysed 1 of 76 tested strains, while ECML-134 lysed 18 strains including the one lysed by EMCL-117. ECML-134 is grown on *E. coli* Ec211/ ECOR-56 / ATCC 35375 which is reported as type O6:H1 while ECML-4 and ECML-117 are grown on type O7157:H7 *E. coli* strains. In total these phage lyse non-O157:H7 *E. coli* strains in less than 9% of tested cases.

Deficiencies: None.

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Temporary Food Tolerance Exemption Petition.
MRID Nos.	None.
Test Material	Lytic monophages for *Escherichia coli* O157:H7; ECML-4, ECML-117 and ECML-134.
Study No.	None given.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Petition Requesting a Temporary Tolerance Exemption from the Requirement of a Tolerance for *E. coli* O157:H7 Specific Bacteriophages used on Food-Contact Surfaces in Food Processing Plants.
Author	None given.
Study Completed	None given.
Study Summary	Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and peer- reviewed literature or analysis of host strain and bacteriophage properties show the host strains are atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes in the bacteriophage, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces. PCR data showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins Stx-1 or Stx-2 was submitted in MRID 481521-01. Data showing that monophage lysed tested non-O157:H7 *E. coli* in less than 9% of instances and did not lyse various *Listeria*, *Salmonella*, *Staphylococcus* or *Pseudomonas* species was submitted in MRID 481521-02.
Classification	**ACCEPTABLE.**
Good Laboratory Practice	Not applicable.

Presence in the Environment:

According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5 \times 10^8$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained $10^3\text{-}10^5$ PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

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- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
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- Børsheim, K.Y. 1993. Native marine bacteriophages. FEMS Microbiol. Ecol. 102, 141–159.
- Calci, K.R., Burkhardt III, W., Watkins, W.D. & Rippey, S.R. 1998. Occurrence of Male-Specific Bacteriophage in Feral and Domestic Animal Wastes, Human Feces, and Human Associated Wastewaters. Appl. Environ. Microbiol. 64, 5027-5029.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. 92, 77–87.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
- Fuhrman, J.A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399, 541-548.
- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127.
- Maranger, R. & Bird, D.E. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. 31, 141–151.
- Noble, R.T. & Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.

- Proctor, L.M. & Fuhrman, J.A. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60–62.
- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.
- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophages are commonly consumed by humans via various foods. In this context, bacteriophages have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophages. For example, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from $3.3\text{-}4.4\text{x}10^{10}$ PFU/100 g of fresh chicken, up to $3.5\text{x}10^{10}$ PFU/100 g of fresh pork, and up to $2.7\text{x}10^{10}$ PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of $10^{1}\text{-}10^{5}$ PFU/100 g meats and up to 10^{5} PFU/g (10^{7} PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/
- Armon, R., Araujo, R., Kott, Y., Lucena, F. and Jofre, J. 1997. Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. J. Appl. Microbiol. 83, 627-633.
- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. 2003.

lsolation and Characterization of *Campylobacter* Bacteriophages from Retail Poultry. Appl. Environ. Microbiol. 69, 4511-4518.
- Gautier, M., Rouault, A., Sommer, P. and Briandet, R. 1995. Occurrence of *Propionibacterium freudenreichii* Bacteriophages in Swiss Cheese. Appl. Environ. Microbiol. 61, 2572-2576.
- Greer, G.G. 2005. Bacteriophage Control of Foodborne Bacteria. J. Food Prot. 68, 1102-1111.
- Kennedy Jr., J.E., Oblinger, J.L. and Bitton, G. 1984. Recovery of Coliphages from Chicken, Pork Sausage and Delicatessen Meats. J. Food Prot. 47, 623-626.
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- Maciorowski, K.G., Pillai, S.D. and Ricke, S.C. 2001. Presence of Bacteriophages in Animal feed as Indicators of fecal Contamination. J. Enciron. Sci. Health B36, 699-708.
- Whitman, P.A. and Marshall, R.T. 1971. Isolation of Psychrophilic Bacteriophage-Host Systems from Refrigerated Food Products. Appl. Microbiol. 22, 220-223.

Health Effects:

Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects (Alisky et al 1998, Sulakvelidze et al 2001). Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from the bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight (Lopez 1975, Ochs et al. 1992). *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication (Bruttin and Brussow 2005).

References:

-Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. 1998. Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15.
- Bruttin, A. & Brussow, H. 2005. Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878.
- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. & Wedgewood, R.J. 1975. Defective antibody response to bacteriophage ΦX174 in Down syndrome. J. Pediatrics 86, 207-211.
- Ochs, H.D., Buckley, R.H., Kobayashi, R.H., Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. & Herchfeld, M.S. 1992. Antibody Responses to Bacteriophage ΦX174 in Patients With Adenosine Deaminase Deficiency. Blood 80, 1163-1171.
-Sulakvelidze, A., Alavidze, Z. & Morris Jr., J.G. 2001. Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659.

Transduction, Lysogeny and Bacteriophage Sequencing:

The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to

absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:

- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Host Range Testing:

Data showing that monophage do not lyse 5 strains of *Listeria monocytogenes*, 5 species of *Salmonella* (*enteritidis*, *typhimurium*, *newport*, *paratyphi* B, *dublin*), 5 strains of *Staphylococcus aureus* or 5 strains of *Pseudomonas aeruginosa* was submitted in MRID 481521-02. Also included was testing of individual monophage for lysis against non-O157:H7 *E. coli* strains. ECML-4 and ECML-117 each lysed 1 of 76 tested strains, while ECML-134 lysed 18 strains including the one lysed by EMCL-117. ECML-134 is grown on *E. coli* Ec211/ ECOR-56 / ATCC 35375 which is reported as type O6:H1 while ECML-4 and ECML-117 are grown on type O7157:H7 *E. coli* strains. In total these phage lyse non-O157:H7 *E. coli* strains in less than 9% of tested cases.

Deficiencies: None.

Fw: link to the FR examples
Dennis Edwards to: Tracy Lantz 09/08/2010 08:40 PM
Cc: Velma Noble

Tracy

See link below for your temp tolerance template. This is a start. I will see what else I can find.

Dennis

Dennis Edwards, Chief
Regulatory Management Branch 1
Antimicrobials Division
703-308-8087
----- Forwarded by Dennis Edwards/DC/USEPA/US on 09/08/2010 08:39 PM -----

From:	Dan Rosenblatt/DC/USEPA/US
To:	Dennis Edwards/DC/USEPA/US@EPA
Date:	09/08/2010 03:06 PM
Subject:	link to the FR examples

http://intranet.epa.gov/oppthome/intrafrs/opptempl.htm#registration

Hi Dennis - Take a look at this link. It's the resource by John Richards around the general FR templates that are done. With a quick glance - I think maybe template #401 might be one that you could use as a starting point. The section 18 template for setting time-limited tolerances is #403 and that also could give you some ideas.

Give me a call if you want to talk through this.

Dan
308-9366

Dennis' link to FR template website attached

(looks like they are intranet

↓

save template to laptop + bring home?

↓

must stay in office this week!

Re: Temporary tolerance for a bacteriophage
Chris Kaczmarek to: Tracy Lantz
Cc: Jonathan Fleuchaus
09/07/2010 12:21 PM

Confidential
Attorney-Client Communication
Attorney Work Product
Pre-Decisional/Deliberative -- Do Not Release Under FOIA Without Further Review

[redacted]

Commercial/financial information may be entitled to confidential

[redacted]

talk to: Denise Greenway John Kough BPPD

conditions of registration may go in EUP

[redacted]

Thanks, Chris!

Chris Kaczmarek

Tracy Lantz Hi Chris, My name is Tracy, I'm in AD and am cu... 09/07/2010 10:45:33 AM

From: Tracy Lantz/DC/USEPA/US
To: Chris Kaczmarek/DC/USEPA/US@EPA
Date: 09/07/2010 10:45 AM
Subject: Temporary tolerance for a bacteriophage

Hi Chris, — 202 564 3909

My name is Tracy, I'm in AD and am currently working on an experimental use permit with a temporary food tolerance for a bacteriophage. This is my first time processing this type of action, so am seeking your advice. I have been directed to you as an OGC contact for this type of action. Would you please send me a similar document that you have worked on that I may use as a template for this action?

Thanks in advance.

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

Decision #: 416027
DP #: (381433)
PRIA
Parent DP #:
Submission #: 852425

DATA PACKAGE BEAN SHEET

Date: 26-Aug-2010

* * * Registration Information * * *

Registration: 74234-EUP-E -

Company: 74234 - INTRALYTIX, INC.

Risk Manager: RM 31 - Velma Noble - (703) 308-6233 Room# PY1 S-8855

Risk Manager Reviewer: Tracy Lantz TLANTZ

Sent Date: ______ Calculated Due Date: 10-Jan-2011 Edited Due Date: ______

Type of Registration: Experimental Use Permit - Sectio

Action Desc: (A520) NEW USE;EUP;

Ingredients: 016432, E. coli 0157:H7 Specific Bateriophages(0%)

* * * Data Package Information * * *

Expedite: ◯ Yes ● No Date Sent: 26-Aug-2010 Due Back: ______

DP Ingredient: 016432, E. coli 0157:H7 Specific Bateriophages

DP Title: ______

CSF Included: ◯ Yes ● No Label Included: ◯ Yes ● No Parent DP #: ______

270 / 189

90 Day Rule Applied

Assigned To		Date In	Date Out
Organization:	AD / PSB	8/26/10	
Team Name:	EET	8/26/10	
Reviewer Name:	Tajah	9/1/10	
Contractor Name:			

Last Possible Science Due Date: 21-Oct-2010

Science Due Date: 2/17/11

Sub Data Package Due Date: 3/3/11

* * * Studies Sent for Review * * *

No Studies

1) Not accepting in lieu of lab (2)

* * * Additional Data Package for this Decision * * *

Can be printed on its own page

* * * Data Package Instructions * * *

Tajah - I apologize for the delay in sending this package. I initially did not realize that there was an efficacy response for review. The January due date is when we have to post the temporary tolerance. We hope to have your review well in advance at that date.

Tracy, Can you ~~arrange~~ schedule a mtg c Dennis, Emily +

RECEIVED 8/26/10

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

September 29, 2010

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 74234-EUP-E, ECP-100,
DP Barcode: 381433

From: Tajah Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

9/29/10

To: Velma Noble PM31/Tracy Lantz
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Intralytix, Inc.
701 East Pratt Street
Baltimore, MD 21202

Formulation from the Label

Active Ingredient(s)	% by wt.
E. coli O t57:H7 (specific Bacteriophages*)	0.00027%
Other Ingredients	99.99973%
Total	100.00000 %

*Comprised of the following monophages: ECML-4, ECML-117, and ECML-134. Nominal titer of ECP-100 is 10^{10} PFU/ml.

I BACKGROUND

In the current submission, the registrant provided response to the Agency's Efficacy Review (dated February 16, 2010). ECP-100 is a new product for use in the control of *E. coli* O157:H7 on food and non-food contact surfaces in processing plants. Data packages have been submitted in request of an Experimental use Permit (EUP). Agency's comments and registrant's responses are provided below.

II USE DIRECTIONS

ECP-100 is for use on food and non-food contact surfaces in food-processing plants. Directions on the proposed label provided the following instructions for the use and preparation of the product:

Prior to application, add 1 part of ECP-100 into a clean container. Then add 9 parts of non-chlorinated water. If water is taken from a chlorinated source, allow the water to sit at room temperature for 24 hours prior to addition to ECP-100. After dilution, the use-solution of working titer of ECP-100 is approximately 10^9 PFU/ml. Apply the ECP-100 use solution by either spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to ECP-100 application. Only use ECP-100 as an adjunct to EPA registered food contact sanitizers. Apply ECP-100 at least 5 minutes prior to using an EPA registered sanitizer following the use-instructions for the EPA registered sanitizer.

III REGISTRANT'S RESPONSES TO AGENCY QUESTIONS

Agency Initial Comment 1. Will the use sites (in the proposed EUP) be separate from other locations were the bacteriophage is not in use? How is efficacy demonstrated/monitored? Are the test use sites chemically sanitized before the bacteriophages are added to surface? What is the contact time for bacteriophage activity? How is the activity neutralized?
Registrant's Response: ECP-100 will not be used separately but only in conjunction with currently registered food contact surface sanitizer products. The objective of the EUP experimental program is to evaluate whether ECP-100, when used as an adjuvant, assists in the reduction of *E. coli* O157:H7 under actual plant conditions. As previously communicated to the Agency, a food company has expressed interest in testing ECP-100 under these conditions (see attachment). The product label for ECP-100 and experimental program will be revised to clearly indicate that ECP-100 is to be used in association with registered food contact surface sanitizer products.
→ Agency's Final Comment: The revised product label and experimental program must be submitted to the Agency.

Agency's Initial Comment 2. For both food contact and non-food contact surfaces, will these surfaces be used for food processing after/before testing begins?
Registrant's Response: The treated surfaces will be used for food processing both before and after testing. As indicated above, ECP-100 will be used as an adjuvant in conjunction with registered food-contact surface sanitizers used by the food-processing

facility. Accordingly, the target food-contact surfaces will be treated both with ECP-100 and a registered food-contact sanitizer.
Agency's Final Comment: No additional information is required.

Agency's Initial Comment 3. How is efficacy demonstrated/monitored?
Registrant's Response: Efficacy will be monitored by sampling treated areas per existing Hazard Analysis and Critical Control Point (HACCP) protocols.
Agency's Final Comment: No additional information is required.

Agency's Initial Comment 4. Are the test use sites chemically sanitized before the bacteriophages are added to surface?
Registrant's Response: The test-sites will be treated with an EPA registered food contact surface sanitizer subsequent to the application of ECP-100. This will be indicated on the revised product label for ECP-100.
Agency's Final Comment: No additional information is required.

Agency's Initial Comment 5. What is the contact time for bacteriophage activity? How is the activity neutralized?
Registrant's Response: The contact time will be approximately 5 minutes. No neutralization step will be necessary since: 1) the bacteriophages will be inactivated by the application of the chemical sanitizer and 2) the phages will naturally inactive over time. Moreover, since the EUP is requesting actual plant trials, only substances that are approved for use on food-contact surfaces can be used as neutralizers.
Agency's Final Comment: Is the 5-minute contact for non-food contact surfaces or food contact surfaces?

Agency's Initial Comment 6. How will pre-treatment concentrations of *E. coli* O157:H7 be determined (can you provide the means by which monitoring is normally conducted)? What is the typical *E. coli* O157:H7 level of contamination? What are the conditions of the use surfaces before exposure to peroxyacetic acid, quaternary ammonium detergents, or sodium hypochlorite and/or bacteriophages?
Registrant's Response: The pre-treatment concentrations of *E. coli* O157:H7 will be determined by the procedures in the facility's HACCP program. However, it should be noted, that food plants are reluctant to disclose contamination levels with any bacterium. Even so, there appears to be a problem with *E. coli* O157:H7, which is why there is an interest in testing ECP-100 under real-world conditions.
Agency's Final Comment: A baseline of typical *E. coli* O157:H7 contamination is critical to determine if use of the product is advantageous. A final report of the experimental data is required

Agency's Initial Comment 7. Surfaces in the processing plant are inevitably contaminated with other microorganisms. If the anticipated use of this product is an "alternative to chemical sanitizers", what is the proposed treatment against other microorganisms?
Registrant's Response: As indicated above, ECP-100 will be used in conjunction with EPA registered food contact surface sanitizers. The sole purpose of ECP-100 is to evaluate whether it can provide additional protection against *E. coli* O157:H7.
Agency's Final Comment: No additional information is required.

Agency's Initial Comment 8. According to the proposed test method, are there pre-cleaning steps? If so, what are the pre-cleaning steps, in detail? Usage of peroxyacetic acid, quaternary ammonium detergents or sodium hypochlorite may be sufficient for the removal of *E. coli* O157:H7. Therefore treatment of the surfaces with bacteriophage may be useless.
Registrant's Response: There are no pre-cleaning steps prior to the application of ECP-100. However, pre-cleaning steps are required prior to the application of the registered chemical sanitizer. The reviewer may be correct that ECP-100 does not provide any additional benefits but the purpose of the experimental program is to determine if ECP-100 does have any value under real-world conditions.
Agency's Final Comment: Anticipating the amount of gross soil and heavy filth, a pre-cleaning step, prior to applying ECP-100, may be appropriate.

Agency's Initial Comment 9. Will resistance be monitored? How many bacteriophages are in the proposed cocktail? Will the same cocktail be used in every location?
Registrant's Response: Regarding resistance, any possible isolates from the test sites will be evaluated in the laboratory for susceptibility/resistance to ECP-100. The cocktail contains three component monophages that has been optimized for host range and efficacy. The same formulation will be used in all test sites.
Agency's Final Comment: No additional information is required.

Agency's Initial Comment 10. Registered food contact sanitizer must demonstrate a 5 $\log_{10}$ reduction in organisms in 30 seconds. Effectiveness of ECP-100 in reducing the titer of *E. coli* O157:H7 with the goal of achieving a minimum of a two-log reduction will not satisfy the Agency's standard.
Registrant's Response: ECP-100 will only be used as an adjunct, not a stand-alone sanitizer. Assuming the experimental program shows that ECP-100 provides additional control against *E. coli* O157:H7, then registration of ECP-100 should be considered. At the present time, Intralytix is only requesting to conduct experimental trials to evaluate whether ECP-100, when [used] as an adjuvant, provides efficacy under real-world conditions. Intralytix doesn't believe that the Agency needs any more efficacy data to approve the EUP since ECP-100 treatment will be followed by a registered sanitizer product.
Agency's Final Comment: The registrant should be aware that issuance of the EUP is not a substitute for registration, and product specific laboratory data will be required to register the product.

IV AGENCY CONCLUSIONS

Adequate information has been provided to support issuance of an Experimental Use Permit (EUP) for ECP-100 to supplement non-food contact and food-contact sanitization in processing plants. The registrant states in the provided responses that a revised label and experimental program is forthcoming to address the cited deficiencies. This revised information must be submitted to the Agency. The results of the EUP must be submitted to the Agency, and any additional modifications must be provided to the Agency as well. Furthermore, data generated from the EUP is not sufficient to support product registration. Laboratory generated efficacy data consistent with the proposed use sites will be required by the Agency to support registration.

IV LABEL COMMENTS

1. The proposed label does not include the appropriate contact time for non-food contact surfaces.

2. The proposed label should include a more detailed explanation regarding the treatment of surfaces with bacteriophages (i.e. some type of sequential process). The use directions for non-food contact surfaces and food contact surfaces have been merged. Use directions must be separated with an appropriate heading designating the use site.

3. On the proposed label, change *E. coli* 0157:H7 to *E. coli* O157:H7.

RESPONSE TO EFFICACY REVIEW FOR ECP-100,
FILE SYMBOL NO. EUP-74234-E

Agency Comment No. 1
Will the use sites (in the proposed EUP) be separate from other locations where the bacteriophage is not in use.

Intralytix Response
ECP-100 will not be used separately but only in conjunction with currently registered food-contact surface sanitizer products. The objective of the EUP experimental program is to evaluate whether ECP-100, when used as an adjuvant, assists in the reduction of *E. coli* 0157:H7 under actual plant conditions. As previously communicated to the Agency, a food company has expressed interest in testing ECP-100 under these conditions (see attachment). The product label for ECP-100 and experimental program will be revised to clearly indicate that ECP-100 is to be used in association with registered food-contact surface sanitizer products.

Agency Comment No. 2
For both food-contact and non-food contact surfaces, will these surfaces be used for food processing after/before testing begins.

Intralytix Response
The treated surfaces will be used for food-processing both before and after testing. As indicated above, ECP-100 will be used as an adjuvant in conjunction with registered food-contact surface sanitizers used by the food-processing facility. Accordingly, the target food-contact surfaces will be treated both with ECP-100 and a registered food-contact sanitizer.

Agency Comment No. 3
How is efficacy demonstrated/monitored?

Intralytix Response
Efficacy will be monitored by sampling treated areas per existing Hazard Analysis and Critical Control Point (HACCP) protocols.

Agency Issue No. 4
Are the test use-sites chemically sanitized before the bacteriophages are added to surface?

Intralytix Response
The test-sites will be treated with an EPA registered food-contact surface sanitizer subsequent to the application of ECP-100. This will be indicated on the revised product label for ECP-100.

Agency Issue No. 5
What is the contact time for bacteriophage activity? How is the activity neutralized?

Intralytix Response
The contact time will be approximately 5 minutes. No neutralization step will be necessary since: 1) the bacteriophages will be inactivated by the application of the chemical sanitizer and 2) the phages will naturally inactive over time. Moreover, since the EUP is requesting actual plant trials, only substances that are approved for use on food-contact surfaces can be used as neutralizers.

Agency Issue No. 6
How will pre-treatment concentrations of *E. coli* 0157:H7 be determined?

Intralytix Response
The pre-treatment concentrations of *E. coli* 0157:H7 will be determined by the procedures in the facility's HAACP program. However, it should be noted, that food plants are very reluctant to disclose contamination levels with any bacterium. Even so, there appears to be a problem with E. coli 0157:H7, which is why there is an interest in testing *ECP*-100 under real-world conditions.

Agency Issue No. 7
Surfaces in processing plants are inevitably contaminated with other microorganisms. If the anticipated use of this product is an "alternative to chemical sanitizers", what is the proposed treatment against other microorganisms?

Intralytix Response
As indicated above, ECP-100 will be used in conjunction with EPA registered food-contact surface sanitizers. The sole purpose of ECP-100 is to evaluate whether it can provide any additional protection against E coli O157:H7.

Agency Issue No. 8
According to the proposed test method, are there pre-cleaning steps? If so, what are the pre-cleaning steps, in detail? Usage of peroxyacetic acid, quaternary ammonium detergents or sodium hypochlorite may be sufficient from the removal of E. coli 0157:H7. Therefore, treatment of the surfaces with bacteriophage may be useless.

Intralytix Response
There are no pre-cleaning steps prior to the application of ECP-100. However, pre-cleaning steps are required prior to the application of the registered chemical sanitizer. The reviewer may be correct that ECP-100 does not provide any additional benefits but the purpose of the experimental program is to determine if ECP-100 does have any value under real-world conditions.

Agency Issue No. 9
Will resistance be monitored? How many bacteriophages are in the proposed cocktail? Will the same cocktail be used in every location?

Intralytix Response
Regarding resistance, any possible isolates from the test sites will be evaluated in the laboratory for susceptibility/resistance to ECP-100. The cocktail contains three component monophages that has been optimized for host range and efficacy. The same formulation will be used in all test sites.

Agency Issue No. 10
Registered food-contact sanitizers must demonstrate at least a 5 log reduction in organisms within 30 seconds. Effectiveness of ECP-100 in reducing the titer of *E. coli* 0157:H7 with the goal of achieving a minimum of a two log reduction will not satisfy the Agency's standard.

Intralytix Response
ECP-100 will only be used as an adjunct, not a stand-alone sanitizer. Assuming the experimental program shows that ECP-100 provides additional control against *E. coli* 0157:H7, then registration of ECP-100 should be considered. At the present time, Intralytix is only requesting to conduct experimental trials to evaluate whether ECP-100, when as an adjuvant, provides efficacy under real-world conditions. Intralytix doesn't believe that the Agency needs any more efficacy data to approve the EUP since ECP-100 treatment will be followed by a registered sanitizer product.

Email Notification of Negotiated Due Date
Pesticide Registration Improvement Act to: lantz.tracy 08/23/2010 06:05 PM
Sent by: DCOPPAPPS01
Please respond to Pesticide Registration

SUBJECT: Notification -- Due Date for Decision #416027 has been re-negotiated

---

Please note: The PRIA Due Date for the following decision has been negotiated:

Decision Number: 416027
Original Due Date: 04/13/2010
Negotiated Due Date: 01/10/2011 ← new due date confirmed
You are a Reviewer assigned to a data package for this decision

---

This is an automatically generated notification message. Please do not reply to this address.

Check on this

74234-EUP-E

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Recommendation of Division Directors
Negotiated Due Dates

Decision#: D 416027 | **Registration#:** 74234-EUP-E | **Petition #:** 9G7585

Fee Category: A520 | **PRIA Decision Time Frame:** 9 months

Submitted by: Velma Noble/Tracy Lantz | **Branch:** RMBI | **Date:** 8/16/10

Company: Intralytix, Inc

Original Due Date: April 13, 2010 | **Proposed New Due Date:** January 10, 2011

Previous Negotiated Due Dates: one (11/23/10

Is the "Fix" in-house? Yes | **If not, date "Fix" expected:**

Issue (describe in detail): Agency identified a number of concerns related to the submitted efficacy data, product characterization and temporary food tolerance exemption. The most critical issue to be addressed is whether this bacteriophage produces toxins, pathogenicity factors or lysogenized phage. Additional information has been provided to the Agency but did not arrive by promised due date of 6/14/10. Information arrived 7/16/10 thus the need for an additional renegotiation.

Summary of Deficiency Type(s): Not Submitted (N) Deficiencies (D)
Product Chemistry: ___ Acute Tox: ___ Efficacy: __D__ Labeling: ____ Other (describe): __D__

Additional data is required for Agency to make a decision to allow Experimental Use Permit and Temporary Tolerance.

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates): The company was first contacted regarding this deficiency by e-mail on 2/24/10. This was followed up with additional e-mail correspondence on 3/2, 3/26, 3/19, 3/27, and a 75 day letter by e-mail on 3/31. A copy of the same letter was sent by USP on 4/1. Registrant submitted additional data on July 16th and would like to renegotiate the due date to January 10, 2011.

"75 Day" Letter sent? ____X____ (Date sent) 3/31/2010 ____No and reason for none?

Rationale for Proposed Due Date:

New due date allows 177 additional days for review of the new data and establishing the temporary tolerance.

Registrant notified that this is the last negotiation? ____Yes ___X_Not Applicable

Approve: ✓ | **Disapprove:**

~~If disapproved, action~~ to be taken:

8-20-10

OD or DOD Signature: [signature] **Date:**

CONCURRENCES

SYMBOL	7510P	7510P	7510P				
SURNAME	[signature]	[signature]	[signature]				
DATE	8/16/10	8/18/10	8/19/2010				

EPA Form 1320-1A (1/90) *Printed on Recycled Paper* OFFICIAL FILE COPY

RE: ECP-100, File Symbol No. 74234-EUP
Eliot Harrison
to:
Tracy Lantz
07/28/2010 11:24 PM
Cc:
Velma Noble
Show Details

Hi Tracy,
I apologize for the delay in getting to this.

On behalf of Intralytix Inc., I am requesting that the PRIA deadline for ECP-100 be extended from November 23, 2010 until January 10, 2011. The reason for the extension is the delay Intralytix encountered in providing information requested by the Agency.

As I mentioned, we also need to schedule a conference call to discuss the efficacy component. We did not provide any additional efficacy data in response to Tajah's review. Please note the following;

-We did clarify in the response that ECP-100 will only be used as an adjuvant with, and prior to the application of, registered chemical food contact sanitizers.
-It is extremely doubtful that ECP-100 will show any activity in the standard food-contact sanitizer test in 30 seconds. By the way, it is my understanding that the 30 second time period was established since ware washing machines typically ran on a 30-second cycle. Actually, FDA indicated to me several years ago that the new machines ran on a shorter cycle and they wanted to reduce the test time below 30 seconds. Other than ware washing, I don't believe there is really a critical reason why food-contact sanitizers need to meet a 30-second time period.
-ECP-100 will probably show 2-3 log kill, against E. coli O157:H7 in a 5 minute lab study.
-The purpose of the EUP is to evaluate whether ECP-100 is effective under real-world conditions.
-My suspicion is that chemical sanitizers work relatively well on food-contact surfaces, in food processing plants, most of the time. However, I believe that plants do have regular contamination problems with pathogens of concern and are very interested in any new technologies, such as phages, that might reduce this contamination.
-My understanding is that food processors are very reluctant to discuss with regulators these contamination problems because of concern the information could become public and have negative consequences. Therefore, it probably will be difficult even to get these companies to release data they gather from real world studies.
-The interest in the Intralytix products comes from major food processors and also from processors/marketers of pet foods. So, I do believe the contamination problem is a real issue.
-So, we really do need to discuss what efficacy data the Agency will need both for the EUP and full registration.

Finally, one of the requests from BPPD is that we provide a tolerance document for the Federal Register (we did submit a Notice of Filing and Tolerance Exemption Petition). I was working on this document but with the new transparency policy you guys probably have a much better idea of what needs to go in the FR document than I do.

Regards,
Eliot

From: Lantz.Tracy@epamail.epa.gov [mailto:Lantz.Tracy@epamail.epa.gov]
Sent: Wed 7/28/2010 5:36 PM
To: Eliot Harrison
Cc: Noble.Velma@epamail.epa.gov
Subject: Re: ECP-100, File Symbol No. 74234-EUP

Are you going to send the new renegotiation? I have been waiting for this in order to keep this application moving. This arrived at the Agency a little more than 30 days late. How about renegotiated for an

additional 40 days, especially since there may be issues as the due dates now fall near Christmas/New Years when many of our staff including those at the docket, etc may well be on leave.
(Embedded image moved to file: pic17013.jpg)

From: "Eliot Harrison" <eharrison@lewisharrison.com>

To: Tracy Lantz/DC/USEPA/US@EPA

Date: 07/20/2010 11:46 AM

Subject: ECP-100, File Symbol No. 74234-E

Hi Tracy,
I did submit the Intralytix EUP info/data last week. When you get a chance, give me a call so we can discuss a time extension.
Hope everything is going well for you.
Regards,
Eliot

Tyson Foods, Inc.

HAND-DELIVERED

Dennis Edwards, Branch Chief
Regulatory Management Branch #1
Antimicrobials Division (7510W)
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA

Dear Mr. Edwards:

As all of us interested in food safety are well aware, there is an industry-wide problem with a number of food-borne pathogens. Despite the diligent use of chemical sanitizers in HACCP programs, the industry still experiences refractory problems with *E. coli* 0157:H7, *Listeria monocytogenes*, *Salmonella*, and several other pathogens.

Tyson Foods, a leader in food safety programs of meat and poultry food products, wishes to support needed efforts for new and novel technologies and developments that continue to improve the safety of our foods. Accordingly, we urge the Agency to expedite approval of the Experimental Use Permit (EUP) that Intralytix recently submitted for the product ECP-100. This product, which is based on Intralytix's bacteriophage technology, is designed to control *E. coli* 0157:H7 on both non-food contact and food contact surfaces. We believe that it is in the best interest of all to support development of technologies such as this that may directly improve the public health and to which the prompt approval by EPA on the subject EUP would contribute to this effort.

ECP-100 offers a new and novel approach to supplement existing 'chemical based' environmental control methods for *E. coli* 0157:H7. Field testing is a critical component to determine if the product is effective under real world conditions. If possible, Tyson's would initiate field testing of ECP-100 beginning on April 1, 2009. I am available by phone to discuss our interest and plans for ECP-100.

Respectfully,

Dean A. Danilson

Dean A. Danilson, PhD
Vice President
Food Safety & Quality Assurance
Tyson Foods, Inc.
605-235-2158

cc: Betty Schackleford, Deputy Division Director
Joan Farrelly-Harrigan, Division Director

RE: ECP-100, File Symbol No. 74234-EUP
Eliot Harrison
to:
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Show Details

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To: Eliot Harrison
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Subject: Re: ECP-100, File Symbol No. 74234-EUP

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From: "Eliot Harrison" <eharrison@lewisharrison.com>

To: Tracy Lantz/DC/USEPA/US@EPA

Date: 07/20/2010 11:46 AM

Subject: ECP-100, File Symbol No. 74234-E

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Eliot Harrison
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Eliot

LEWIS & HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

July 14, 2010

Sent data to BPPD 7/29/10

promised this info by 6/14 arrived 7/16

Velma Noble, Product Manager (31)
Regulatory Management Branch No. I
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202

Eliot will send new renegotiation for addition 30 days on Monday

8/20/10 conv. w/ Eliot, Velma, Denois we would like to discuss a performance standard for this product 2-3 log reduction in 5 minutes. they will come in to discuss w/ Tajeh et al.

re: **Application for Experimental Use Permit**
Product: ECP-100™
EPA File Symbol No. 74234-EUP-E
OPP Decision Number: D416027
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
Response to Antimicrobials Division (AD) Efficacy Review and Biopesticide and Pollution Prevention (BPPD) Review

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am responding to the AD efficacy review and the BPPD review for the ECP-100 Experimental Use Permit (EUP). Please find enclosed responses to each review. In addition, two additional studies that address issues raised in the BPPD review are enclosed.

If you have any questions about this submission, please contact me at (202) 393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com.

↓ He will request a mtg. w/ us + efficacy

Sincerely,

Eliot Harrison
Agent for Intralytix

LEWIS &
HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

July 14, 2010

Velma Noble, Product Manager (31)
Regulatory Management Branch No. 1
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202

re: **Application for Experimental Use Permit**
Product: ECP-100™
File Symbol No. 74234-EUP-E
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
Data Transmittal Letter for Studies Supporting Experimental Use Permit

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am submitting three (3) copies of the following studies in support of the Experimental Use Permit (EUP) application for ECP-100. These studies are being submitted in response to the Biopesticides and Pollution Prevention (BPPD) review of ECP-100.

- Volume 1 of 2
  Tests for the Presence of Shiga Toxin Genes Stx-1 and Stx-2 in Ec211, or ATCC 35375
  MRID# **48152101**

- Volume 2 of 2
  Lytic Activity of Component Monophages
  MRID# **48152102**

If you have any questions about this submission, please contact me at (202) 393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com.

Sincerely,

Eliot Harrison
Agent for Intralytix

Manufacturing process information may be entitled to confidential treatment

Inert ingredient information may be entitled to confidential treatment

RESPONSE TO BPPD REVIEW FOR ECP-100,
FILE SYMBOL NO. EUP-74234-E

Agency Comment No. 1
Data or peer-reviewed references should be provided showing that the host bacterium E. coli Ec211/ECOR-56/ATC 35373 does not produce shigatoxins.

Intralytix Response
To address this issue, Intralytix is submitting the following study "Test for the Presence of Shiga Toxin Genes, Stx-1 and Stx-2 in Ec211, or ATCC 35375.

Agency Comment No. 2
A revised CSF should be provided listing endotoxin as a contaminant, nominal concentrations of all inert ingredients, [redacted] is listed in the MSDS but not listed on the CSF; minimum PFU/ml for each monophage in ECP-100 (consistent with the manufacturing process).

Intralytix Response
A revised CSF is attached. On the revised CSF: 1) [redacted] is listed as an impurity; 2) [redacted] is included as one of the ingredients in [redacted] 3) the minimum PFU/ml for each monophage in ECP-100 is listed. The nominal concentration for PBS is included but Intralytix does not have the nominal concentrations for each component of PBS. It is our understanding that the Agency typically doesn't require nominal concentrations for components of mixtures. Instead, ranges of the individual has been acceptable.

Agency Comment No. 3
Remove the word specific from the active ingredient description or provide supporting tests for review.

Intralytix Response
Intralytix is submitting the study "Lytic Activity of Component Monophages", which shows that relatively few non- *E. coli* 0157:H7 strains are lysed and that no non-E coli strains are lysed.

Agency Comment No. 4
A label matching the revised CSF should be provided with clear usage and dilution instructions.

Intralytix Response
A revised label is included with this submission.

Decision #: 416027
DP #: (369678)
PRIA
Parent DP #:
Submission #: 852425

DATA PACKAGE BEAN SHEET

Date: 29-Jul-2010

* * * Registration Information * * *

Registration: 74234-EUP-E -

Company: 74234 - INTRALYTIX, INC.

Risk Manager: RM 31 - Velma Noble - (703) 308-6233 Room# PY1 S-8855

Risk Manager Reviewer: Jacqueline Campbell-McFarlane JMCFAR02

Sent Date: _____ Calculated Due Date: 23-Nov-20f0 Edited Due Date: _____

Type of Registration: Experimental Use Permit - Sectio

Action Desc: (A520) NEW USE;EUP;

Ingredients: 016432, E. coli 0f57:H7 Specific Bateriophages(0%)

* * * Data Package Information * * *

Expedite: ● Yes ○ No Date Sent: 28-Sep-2009 Due Back: _____

DP Ingredient: _____

DP Title: Toxicology Evaluation

CSF Included: ● Yes ○ No Label Included: ● Yes ○ No Parent DP #: _____

	Assigned To	Date In	Date Out		
Organization:	BPPD / MPB			Last Possible Science Due Date:	03-Sep-2010
Team Name:	MPB Health & Characterization			Science Due Date:	
Reviewer Name:				Sub Data Package Due Date:	
Contractor Name:					

* * * Studies Sent for Review * * *

Printed on Page 2

* * * Additional Data Package for this Decision * * *

Can be printed on its own page

* * * Data Package Instructions * * *

EXPERIMENTAL USE PERMIT: E. coli 0157:H7 bacteriophage pesticide for treatment of food contact surfaces in food processing plants.

Please reivew Toxicology Waiver Requests and Discussion of Saftey Issues, MRID Nos 47786803

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Recommendation of Division Directors Negotiated Due Dates		
Decision#: D 416027	Registration#: 74234-EUP-E	Petition #:
Fee Category: A520	PRIA Decision Time Frame: 9 months	
Submitted by: Velma Noble/Tracy Lantz	Branch: RMBI	Date: 4/8/10
Company: Intralytix, Inc		
Original Due Date: April 13, 2010	Proposed New Due Date: November 23, 2010	
Previous Negotiated Due Dates: none		
Is the "Fix" in-house? No	If not, date "Fix" expected: June 14, 2010	

Issue (describe in detail): Agency identified a number of concerns related to the submitted efficacy data, product characterization and temporary food tolerance exemption. The most critical issue to be addressed is whether this bacteriophage produces toxins, pathogenicity factors or lysogenized phage. Such screening has not been provided to the Agency

Summary of Deficiency Type(s): Not Submitted (N) Deficiencies (D)
Product Chemistry: ___ Acute Tox: ___ Efficacy: __D__ Labeling: ____ Other (describe): __D__

Additional data is required for Agency to make a decision to allow Experimental Use Permit and Temporary Tolerance.

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates): The company was first contacted regarding this deficiency by e-mail on 2/24/10. This was followed up with additional e-mail correspondence on 3/2, 3/26, 3/19, 3/27, and a 75 day letter by e-mail on 3/31. A copy of the same letter was sent by USP on 4/1. Registrant responded on April 6th indicating that they will send the additional data on June 14th and would like to a renegotiate the due date to Nov. 23, 2010.

"75 Day" Letter sent? ____X____ (Date sent) 3/31/2010 ____No and reason for none?

Rationale for Proposed Due Date:

New due date allows 160 additional days for review of the new data and establishing the temporary tolerance.

Registrant notified that this is the last negotiation? ____Yes ___X_Not Applicable

Approve: √ Disapprove:

If disapproved, action to be taken:

~~OD or DOD~~ Signature: Date: 4-13-10

CONCURRENCES								
SYMBOL	7510P	7510P	7510P					
SURNAME	Sjerk	Edwards	Hamilton Farrell					
DATE	4/8/10	4/8/10	4/8/2010					

LEWIS &
HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

April 6, 2010

Velma Noble, Product Manager (31)
Regulatory Management Branch No. 1
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
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OPP Decision Number: D416027
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
Extension of PRIA Due Date
Agency e-mail Dated March 31, 2010

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am responding to the e-mail from Tracy Lantz to me dated March 31, 2010. The e-mail requested that Intralytix provide a date for submitting the additional information/data requested in the science review for the subject EUP and an extension of the PRIA due date. The PRIA due date needs to be extended to allow the Agency time to review the additional data/information and to establish a temporary tolerance for the active ingredient in ECP-100. Accordingly, please note the following:

- Intralytix will submit the additional information/data requested in the science reviews by June 14, 2010.
- Intraltyix is requesting that the PRIA due date be extended until November 23, 2010.

If you have any questions about this submission, please contact me at (202) 393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com.

Sincerely,

Eliot Harrison
Agent for Intralytix, Inc.

ECP-100
Eliot Harrison to: Tracy Lantz 04/07/2010 12:27 PM
Cc: Velma Noble, Dennis Edwards

Hi Tracy,
Attached is a revised extension letter. As per Joel G's request, I will send in the FR Notice for the tolerance exemption with the rest of the information/data requested.

Eliot ECP-100.ext_20100407112149.pdf

info for EUP renegotiation

LEWIS & HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

April 6, 2010

Velma Noble, Product Manager (31)
Regulatory Management Branch No. 1
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202

re: **Application for Experimental Use Permit**
Product: ECP-100™
EPA File Symbol No. 74234-EUP-E
OPP Decision Number: D416027
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
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Sincerely,

Eliot Harrison
Agent for Intralytix, Inc.

UNI[illegible] STATES ENVIRONMENTAL PROTECT[illegible] AGENCY

APR - 1 2010

OPP Decision Number D416027

Eliot Harrison
Agent for Intralytix, Inc.
Lewis and Harrison
122 C Street, N.W., Suite 740
Washington, DC 20001

Subject: Application for Experimental Use Permit
Product Name: ECP-100
EPA File Symbol: 74234-EUP-E
Applications Dated: May 30, 2009
EPA Receipt Date: June 22, 2009

Dear Mr. Harrison:

Our records indicate that the decision review period for EPA to make a determination regarding the above referenced application ends on April 13, 2010 as pursuant to the Pesticide Registration Improvement Act (PRIA). The application has been determined, pursuant to 40 CFR 152.105, not to be sufficiently complete to process; therefore, the application is considered deficient. Your options under 40 CFR 152.105 and section 33 of FIFRA are addressed separately because each involves a different timeframe and set of options for responding to this letter. Please ensure that you consider each of the sections below in determining how and when you respond to this letter.

40 CFR 152.105:

Pursuant to 40 CFR 152.105, you are allowed 75 days from the date of this letter to provide a response concerning the deficiencies listed in this letter. Your response may include making corrections to complete the application, or notifying the Agency of the date on which you expect to complete the application, or withdrawing your application. If you do not respond to this letter within 75 days or if you respond with a date on which you expect to complete the application but fail to meet that scheduled date, the Agency will treat the application as if you had withdrawn it.

CONCURRENCES								
SYMBOL	7510P							
SURNAME	S Funk							
DATE	3/31/10							

Address the following deficiencies:

Efficacy Data

The submitted information regarding your proposed field study has been reviewed. You must address the questions listed below. The review in its entirety was sent to you via e-mail on 2/24/10.

Resolution of the issues below is required.

1. Will the use sites (in the proposed EUP) be separate from other locations were the bacteriophage is not in use? For both food contact and non-food contact surfaces, will these surfaces be used for food processing after/before testing begins? How is efficacy demonstrated/monitored? Are the test use sites chemically sanitized before the bacteriophages are added to surface? What is the contact time for bacteriophage activity? How is the activity neutralized?

2. How will pre-treatment concentrations of E. coli O157:H7 be determined (can you provide the means by which monitoring is normally conducted)? What is the typical E. coli O157:H7 level of contamination? What are the conditions of the use surfaces before exposure to peroxyacetic acid, quaternary ammonium detergents, or sodium hypochlorite and/or bacteriophages?

3. According to the proposed test method, are there pre-cleaning steps? If so, what are the pre-cleaning steps, in detail? Usage of peroxyacetic acid, quaternary ammonium detergents or sodium hypochlorite may be sufficient for the removal of E. coli O157:H7. Therefore treatment of the surfaces with bacteriophage may be useless.

4. Surfaces in the processing plant are inevitably contaminated with other microorganisms. If the anticipated use of this product is an "alternative to chemical sanitizers", what is the proposed treatment against other microorganisms?

5. Will resistance be monitored? How many bacteriophages are in the proposed cocktail? Will the same cocktail be used in every location?

6. Registered food contact sanitizer must demonstrate a 5 $\log_{10}$ reduction in organisms in 30 seconds. Effectiveness of ECP-100 in reducing the titer of E. coli O157:H7 with the goal of achieving a minimum of a two-log reduction will not satisfy the Agency's standard.

7. The EUP must be supported by laboratory protocol and data. Laboratory data were mentioned, but not provided to demonstrate efficacy of the product in controlled situations.

CONCURRENCES								
SYMBOL								
SURNAME								
DATE								

Product Characterization and Temporary Food Tolerance Exemption

The submitted studies are both **Supplemental but Upgradeable**. The review in its entirety was sent to you via e-mail on 2/24/10. Please see that communication for full details. The major issues at this point are as follows:

The bacterial cultures used to produce bacteriophage for pesticide use must be screened to ensure that these do not produce toxins, pathogenicity factors or lysogenized phage. Using non-toxigenic and non-pathogenic bacteria free of bacteriophage for production of the pesticide eliminates most of the risk concerns – if this cannot be done then devise screening for each batch to ensure no bacterial genes are carried by the phage produced.

Specific for E. coli bacteriophage: Submit PCR, multiplex PCR or full genome sequencing showing that the bacterial cultures do not contain genes for stx1, stx2 or eae. This will address issues associated with the Agency risk assessment. This information is necessary as part of the product characterization and will be used to qualify rationale for any waiver requests. If these genes are found the Agency will revisit the waiver requests and other issues associated with the risk assessment and food safety.

FIFRA Section 33/PRIA:

This application is also subject to a deadline for making a determination on the application under FIFRA Section 33, Pesticide Registration Service Fees, established under PRIA. The time frame for the Agency to make a determination on this application ends on April 13, 2010. Because the deadline for the agency to make a determination on this application expires before the end of the 75 days you have to respond to the deficiencies noted above, you have the following three options:

> **1. Establish a new due date.** You may resolve the issues identified in this letter by submitting a reply to the Agency by April 6, 2010 with information as how you plan to address these deficiencies. Please include your proposed re-negotiated PRIA due date and the date you expect to submit the fix at this time. Your re-negotiated PRIA due date must include the date that you expect to submit the fix plus an additional 160 days beyond the date at which you expect the fix to arrive. This will allow time for Agency review and the establishment of a temporary tolerance and experimental use permit. If no other issues arise as a result of your response to this letter or during our review process, and the information is found to be acceptable, it is the Agency's expectation that resolution of the deficiencies will result in the granting of your application.

2. **Withdraw the application.** Alternatively, you may notify us not later than April 6, 2010 that you are withdrawing your application. As noted above, withdrawal concludes the Agency's review of your application; however, you may resubmit your application after the deficiencies have been addressed. Should you choose to resubmit your application, it would be subject to a new deadline for making a determination on your application and a new registration service fee.

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Please respond to this letter by **April 6, 2010** by contacting Tracy Lantz by telephone, (703) 308-6415, or by e-mail at Lantz.tracy@epa.gov and Velma Noble by telephone at (703) 308-6233 or by e-mail at Noble.velma@epa.gov with a response and for any questions concerning this letter. When submitting information or data in response to this letter, a copy of this letter should accompany the submission to facilitate processing.

Sincerely,

Velma Noble
Product Manager 31
Regulatory Management Branch I
Antimicrobials Division (7510P)

7510P: T.Lantz:3/31/10:74234-EUP-E less than 75 deficiency letter

75 day deficiency letter for 74234-EUP-E
Tracy Lantz to: Eliot Harrison 03/31/20 t0 05:34 PM
Cc: Velma Noble

You will receive a hard copy of this letter in the mail. Please reply by April 6th.

Thanks

OPP Decision Number D416027

Eliot Harrison
Agent for Intralytix, Inc.
Lewis and Harrison
122 C Street, N.W., Suite 740
Washington, DC 20001

Subject: Application for Experimental Use Permit
Product Name: ECP-100
EPA File Symbol: 74234-EUP-E
Applications Dated: May 30, 2009
EPA Receipt Date: June 22, 2009

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Tracy Lantz

Tracy Lantz
Regulatory Team 31
Antimicrobials Division
U. S. Environmental Protection Agency
Phone: (703) 308-6415
FAX: (703) 308-8481

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

February 16, 2010

MEMORANDUM

Subject: Experimental Use Permit Efficacy Review for EPA File Symbol 74234-E, ECP-100; DP Barcode: 368303

From: Tajah Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

Thru: Emily Mitchell, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Velma Noble PM 31/ Jacqueline McFarland
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Intralytix, Inc.
701 East Pratt Street
Baltimore, MD 21202

Formulation from the Label:

Active Ingredient(s)	% by wt.
E. coli O157:H7-Specific Bacteriophages (10^{10} PFU/ml)	0.00027%
Other Ingredients	99.99973%
Total	100.000 %

I BACKGROUND

ECP-100 is a new product for use in the control of *E. coli* O157:H7 on food and non-food contact surfaces in food processing plants. The current data package was submitted to request Experimental Use Permit (EUP) in the designated location. Laboratory data against LMP-100 (bacteriophages specific to Listeria monocytogenes) was previously submitted to demonstrate effectiveness on non-food contact surfaces in food processing plants. Current data package contains information regarding a proposed field study (The data package lacked the required registrant's letter, detailing the purpose of the submission and background information), and the proposed label. Within the text of the proposed study the following background information is provided:

> "Although bacteriophage have [sic] been used extensively as human therapeutics, they have not been previously employed to control environmental pathogens. The purpose of this application for an Experimental Use Permit is to permit assessment of the suitability of bacteriophage as a replacement for the chemical sanitizers currently employed to control E. coli O157:H7 in the beef processing environment that include quaternary ammonium detergents, citric acid, peroxyacetic acid, and sodium hypochlorite."

II USE DIRECTIONS

Directions on the proposed label provided the following instructions for the use and preparation of ECP-100: Prior to application, dilute ECP-100 in carboys in non-chlorinated water to a working concentration of 109 PFU/ml. Apply the use-solution by either spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to ECP-100 application.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

There exist no Agency standards for the proposed uses.

IV SYNOPSIS OF PROPOSED STUDY

Location and Size of Trials

Trials are planned for beef processing plants, in the states of NE, WA, TX, KA, IA, and IL belonging to and operated by Tyson Fresh Meats, Inc. Use areas in the trial will include both food contact and non-food contact surfaces, including floors, walls, areas around drains and gratings, non-food contact equipment as well as tables, conveyors, slicing equipment and related food contact surfaces. Up to 150,000 square feet of interior space will be treated per processing plants.

Participants and Cooperators

Study Director—Alexander Sulakvelidze, Ph.D.
Intralytix, Inc.
701 E. Pratt Street
Baltimore, MD 21202

Cooperator—Dean Danilson, Ph.D.
Vice-President, Food Safety and Quality Assurance
Tyson Fresh Meats, Inc.
800 Stevens Port Drive
Dakota Dunes, SD 57409

States and Acreages

The trials will be conducted from June 1, 2009 until June 1, 2011, in the states identified in the chart. The scope of the trials, on an annual basis, is summarized as well.

State	Locations Planned	Interior Square Footage Planned	Gallons of Formulation	Pounds of Active Ingredient
NE	2	300,000	3600	0.098
WA	1	150,000	f800	0.049
TX	1	150,000	1800	0.049
KA	2	300,000	3600	0.098
IA	1	150,000	1800	0.049
IL	1	150,000	1800	0.049
Total	8	1,200,000	14,400	0.392

The current plans of Intralytix are to conduct trials at a single Tyson Fresh Meats, Inc., facility in Dakota City, NE. Other sites may be added depending upon the success of the results obtained. However, depending on plant availability, trials may have to be rearranged. Nonetheless, the total number of trials planned, surface treated, gallons of formulation and pounds of active ingredient will remain the same.

Program Details

General Description

ECP-100 is a preparation of lytic bacteriophage highly specific for *E. coli* O157:H7. When ECP-100 bacteriophage encounter *E. coli* O157:H7, they sequentially attach to the bacterial cell surface, inject their DNA into the bacterium, replicate within the bacterial host, and liberate the phage progeny by lysing the bacterium, rendering it definitively and permanently incapable of causing subsequent food-borne illness. Previous laboratory experiments under controlled conditions have shown that *E. coli* O157:H7 bacteriophage are capable of achieving substantial reduction of *E. coli* O157:H7 under experimental conditions.

Applications

All experimental applications will be performed under direct supervision of Intralytix personnel. Intralytix will furnish bacteriophage preparations in sterile 500 ml plastic bottles containing a concentrate of *E. coli* O157:H7 bacteriophage ECP-100 with a titer of approximately 10^{10} PFU/ml. At the site of application, the concentrate will be diluted in carboys in either non-chlorinated water or phosphate-buffered saline to a working concentration of 10^{9} PFU/ml. Working *E. coli* O157:H7 bacteriophage solutions will be applied either by spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to bacteriophage application. The final bacteriophage concentration on treated surfaces is estimated to be 10^{9} to 10^{10} PFU/ft^2 at the time of application.

Objectives

An Experimental Use Permit will enable Intralytix to determine if the efficacy of *E. coli* O157:H7 bacteriophage ECP-100 in reducing or eliminating *E. coli* O157:H7 contamination of surfaces in controlled laboratory experiments can be replicated under field conditions in a working beef processing plant environment. The specific objectives of the EUP program include the determination of the following:

- Effectiveness of ECP-100 in reducing the titer of *E. coli* O157:H7 with the goal of achieving a minimum of a two-log reduction.
- Optimization of application and use under commercial conditions and standards.
- Performance using different modes and schedules of applications.
- Comparison with existing *E. coli* O157:H7 control measures.
- Suitability of ECP-100 as a replacement for existing control measures for *E. coli* O157:H7 that presently include chlorine, peroxyacetic acid, quaternary ammonium compounds. Removal of these agents from the beef processing environment is desirable because of hazards posed to workers and the potential for environmental damage.

Assessment of Results

Perdue personnel will apply ECP-100 to interior surfaces and non-food contact equipment in accordance with Intralytix protocols. In general, ECP-100 will be applied at a density of 12 ml/sq^2 once per day. Prior to initiation of treatment, *E. coli* O157:H7 contamination will be assessed by the routine measures used by Tyson Fresh Meats to test for *E. coli* O157:H7 species as part of HACCP program. All microbial assays are performed under the guidelines of both the Association of Official Analytical Chemists and the Microbiology Laboratory Guide of United States Department of Agriculture.

Under the requested EUP, ECP-100 will be evaluated following use of either peroxyacetic acid, quaternary ammonium detergents, or sodium hypochlorite, and will be tested without use of any of these agents. Rates of *E. coli* O157:H7 positivity will be monitored by standard Tyson Fresh Meats monitoring procedures. The expectation is that the frequency of positive *E. coli* O157:H7 cultures will be the same or less than the rate obtained with existing control mechanisms.

Project Justification

E. coli O157:H7 causes significant disease in susceptible individuals, and has been responsible for recalls totaling millions of pound [sic]. Current methods of control involve treatment of surfaces in the processing plant with peroxyacetic acid, sodium hypochlorite at 200 ppm, and quaternary ammonium detergents. ECP-100 offers a biological control alternative to these chemicals sanitizers.

V CONCLUSIONS

Resolution of the issues below is required.

1. Will the use sites (in the proposed EUP) be separate from other locations were the bacteriophage is not in use? For both food contact and non-food contact surfaces, will these surfaces be used for food processing after/before testing begins? How is efficacy demonstrated/monitored? Are the test use sites chemically sanitized before the bacteriophages are added to surface? What is the contact time for bacteriophage activity? How is the activity neutralized?

2. How will pre-treatment concentrations of *E. coli* O157:H7 be determined (can you provide the means by which monitoring is normally conducted)? What is the typical E. coli O157:H7 level of contamination? What are the conditions of the use surfaces before exposure to peroxyacetic acid, quaternary ammonium detergents, or sodium hypochlorite and/or bacteriophages?

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4. Surfaces in the processing plant are inevitably contaminated with other microorganisms. If the anticipated use of this product is an "alternative to chemical sanitizers", what is the proposed treatment against other microorganisms?

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6. Registered food contact sanitizer must demonstrate a 5 $\log_{10}$ reduction in organisms in 30 seconds. Effectiveness of ECP-100 in reducing the titer of *E. coli* O157:H7 with the goal of achieving a minimum of a two-log reduction will not satisfy the Agency's standard.

7. The EUP must be supported by laboratory protocol and data. Laboratory data were mentioned, but not provided to demonstrate efficacy of the product in controlled situations.

Re: Fw: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

Joel Gagliardi to: Tracy Lantz 03/30/2010 11:32 AM

Cc: Dennis Edwards, Sanyvette Williams, Velma Noble

Tracy,
I spoke with Eliot Herman about some issues hoping to resolve them earlier.
He sent along some citations that were supposed to show the serology of the *E. coli* used to grow the phage. Unfortunately, they did not.
He is requesting a meeting but perhaps you can just ask for an explanation or proposed agenda and we may not need one (i.e. see end of message).

I put together a draft risk assessment plan for bacteriophage in general.
Utilizing the microbial data requirements and test guidelines, since bacteriophage are not expected to cause infection or effects to any other organism than a limited range of target bacteria, registrants can seek waiver requests for most or all of the health effects studies. The main requirement for viruses is cell culture testing - though that is meant for viruses that infect animals - which bacteriophage do not so they can seek a waiver for this also.
As written, we have no direct guidance specific to bacteriophage so we have to adapt what we have for an appropriate risk assessment.
Product characterization is where we need detailed information - 885.1100, 885.1200, 885.1300, 885.1400 and 885.1500.
Since bacteriophage are already present in soil and water at very high levels (essentially there likely are more bacteriophage than bacteria in the environment) and there is a lot of literature around showing this, most, and maybe all, nontarget data can be addressed by waivers also.

However, there are issues with bacteriophage that can carry toxin and pathogenicity genes, and essentially convert a non-pathogen to a pathogen. This is how *E. coli* O157:H7 was formed, acquiring stx1, stx2 and eae genes plus a host of others, from *Shigella*. There is also a report of a plant bacterium acquiring a toxin from bacteriophage that kills grazing cattle. These are just a few examples.
So, issues specific to bacteriophage and health of humans or nontarget organisms are as follows:
Potential to carry genes that code for toxins or pathogenicity factors should be ruled out. Note research in the area of "pathogenicity islands" that has been published.

Some approaches: Use only phage that lyse the host and not those prone to lysogeny.
Use only phage that have a narrow host range.
Potentially, screen each batch for bacterial genes, though it is likely difficult to design a test or analyze the results.
Use bacteria that lack toxins and pathogenicity factors to grow pesticidal bacteriophage.
Use bacteria that lack lysogenized bacteriophage to grow pesticidal bacteriophage.

The major issue I need addressed is:
The bacterial cultures used to produce bacteriophage for pesticide use should be screened to ensure that these do not produce toxins, pathogenicity factors or lysogenized phage. Using non-toxigenic and non-pathogenic bacteria free of bacteriophage for production of the pesticide removes a lot of risk concerns - otherwise, we have to devise screening for each batch to ensure no bacterial genes are carried by the phage produced.

Specific for *E. coli* bacteriophage: The registrant can submit PCR, multiplex PCR or full genome sequencing showing that their bacterial cultures do not contain genes for stx1, stx2 or *eae*. That will address the entire remaining issues for our EUP risk assessment. This is part of product characterization and will be used to qualify rationale for any waiver requests. They already submitted sequences for their bacteriophage and did not find these genes.

If they do find these genes we will have to revisit several issues (and waiver requests) for risk assessment

and food safety.

Joel

****
Joel V. Gagliardi, Ph.D.
U.S. Environmental Protection Agency, Mailcode 7511-P
OPPTS, OPP, BPPD, Microbial Pesticides Branch
1200 Pennsylvania Avenue, NW
Washington, DC 20460

703-308-0116 - phone / 703-305-0118 or 703-308-7026 - fax
http://www.epa.gov/pesticides/biopesticides
****

Tracy Lantz Hi Joel, The notes below are in response to your... 03/29/2010 05:43:54 PM

From: Tracy Lantz/DC/USEPA/US
To: Joel Gagliardi/DC/USEPA/US@EPA
Cc: Sanyvette Williams/DC/USEPA/US@EPA, Dennis Edwards/DC/USEPA/US@EPA, Velma Noble/DC/USEPA/US@EPA
Date: 03/29/2010 05:43 PM
Subject: Fw: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

[attachment "[Untitled].pdf" deleted by Joel Gagliardi/DC/USEPA/US]

Hi Joel,

The notes below are in response to your review attached above. Please give me an indication as to whether what he is proposing would address your needs for the upgradable study. In addition, please let me know how much time you would need for your review.

When I do the renegotiation, I would also have to allow time for all the steps that need to occur subsequent to that at the AD end. We have not started the tolerance write up and will need to go thru the Office of General Counsel (OGC) to do so, this will likely take quite a bit a time. Once we get concurrence from OGC we will need to send it over to the Federal Register for type setting etc. I also do not know if Steve Owens would need to be involved.

I would suggest that AD needs at least 90 days on top of whatever time BPPD needs. Based on their proposed renegotiation, we would need your review by the beginning of May. Will you be able to do that, or do I need to ask for a longer time frame?

----- Forwarded by Tracy Lantz/DC/USEPA/US on 03/29/2010 05:10 PM -----

From: "Eliot Harrison" <eharrison@lewisharrison.com>
To: Tracy Lantz/DC/USEPA/US@EPA, Joel Gagliardi/DC/USEPA/US@EPA
Cc: Dennis Edwards/DC/USEPA/US@EPA, Velma Noble/DC/USEPA/US@EPA, Sanyvette Williams/DC/USEPA/US@EPA
Date: 03/29/2010 04:33 PM
Subject: RE: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

Hi Tracy, Joel:

We have reviewed the BPPD and AD reviews for the ECP-100 EUP and have the following comments:

1. The only substantive issue in terms of timing is the shigatoxins issue - "data or peer-reviewed references should be provided showing that the host bacterium E. coli EC211/ECOR-56/ATCC 35375 does not produce shigatoxins". The pertinent information we have on this bacterium is the ATCC classification as "Biosafety Level 1" (not known to cause disease in healthy adult humans) and the paper from Ochman showing that this is a natural E. coli isolate (attached). Will this be enough for the EUP? If not, a significant amount of time will be needed to develop the shigatoxin data.

2. The other information requested in the BPPD review (revised CSF and label; host-range testing of non-0157:H7 E coli and bacteria other than E coli) can be provided within 2 weeks.

3. Can you please clarify the statement in the BPPD review, "a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register". My understanding is that AD will put together the temporary tolerance exemption notice for the FR based on BPPD's reviews.

4. I believe that the outstanding efficacy issues can be addressed by submitting a revised Experimental Program and label that clearly indicates that ECP-100 is an adjuvant and is not a replacement for chemical sanitizers. As indicated in the attachment, food processors are interested in evaluating ECP-100, under real-world conditions, to see if it can assist in the reduction of E. coli 0157:H7 levels.

If issue 1 (shigatoxins) can be addressed by the attached documents, then Intralytix is requesting that the PRIA time-frame be extended until August 1, 2010. The additional information mentioned above will be submitted by April 10, 2010. If additional data is needed to address the shigatoxin issue, I will need to get back to you later this week with a different time-frame since an extended time-period will probably be necessary to develop the data.

Regards,
Eliot

-----Original Message-----
From: Lantz.Tracy@epamail.epa.gov [mailto:Lantz.Tracy@epamail.epa.gov]
Sent: Saturday, March 27, 2010 12:06 PM
To: Eliot Harrison
Cc: Edwards.Dennis@epamail.epa.gov; Noble.Velma@epamail.epa.gov; Williams.Sanyvette@epamail.epa.gov
Subject: Fw: ECP-100 EUP 74234-EUP-E waiting for PRIA fix

Just a reminder that you had promised your fix by Monday, March 29th. Please send a copy to front end processing and a pdf copy to my via e-mail so I that I can speak to BPPD about their review time frame.

----- Forwarded by Tracy Lantz/DC/USEPA/US on 03/27/2010 12:03 PM -----

From: Tracy Lantz/DC/USEPA/US

To: "Eliot Harrison" <eharrison@lewisharrison.com>

Cc: Dennis Edwards/DC/USEPA/US@EPA, Velma Noble/DC/USEPA/US@EPA, Sanyvette Williams/DC/USEPA/US@EPA

Date: 03/19/2010 04:09 PM

Subject: Re: ECP-100 EUP 74234-EUP-E

I would need to speak with BPPD as to how much time they would need for their review.

Then we would also have to allow time for all the steps that need to occur subsequent to that at the AD end. We have not started the tolerance write up and will need to go thru the Office of General Counsel (OGC) to do so, this will likely take quite a bit a time. Once we get concurrence from OGC we will need to send it over to the Federal Register for type setting etc. I also do not know if Steve Owens would need to be involved.

I would suggest that AD needs at least 90 days on top of whatever time BPPD needs. The current PRIA due date is 4/13/10. I would suggest that you not send in a formal renegotiation until we have your fix and have talked to BPPD. The reviewer has already moved on and is currently involved in other projects and would need to finish those reviews before they could pick up this review again.

From: "Eliot Harrison" <eharrison@lewisharrison.com>

To: Tracy Lantz/DC/USEPA/US@EPA

Date: 03/19/2010 03:34 PM

Subject: ECP-100 EUP

Hi Tracy,
I apologize for not getting back to you but I was waiting for the Intralytix folks to let me know if they had the data to respond to the BPPD review or needed time to develop data. Supposedly, they have the data and I should have it next Friday. So, it should be submitted by March 29. I don't think it will be very much to review but I think a 60 day time extension may be needed. If this sound reasonable, I will send you a PRIA extension on Monday. By the way, what is the PRIA due date?
Regards,
Eliot

[attachment "ECP-100_20100329152243.pdf" deleted by Joel Gagliardi/DC/USEPA/US]

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

JAN 12 2010

MEMORANDUM

***** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION *****

SUBJECT: Experimental Use Permit and Temporary Food Tolerance Exemption for ECP-100™ containing three lytic monophages specific for *E. coli* O157:H7.

TO: Jacqueline Campbell-McFarlane
Regulatory Management Branch I,
Antimicrobials Division (7510-P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511-P)

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511-P)

ACTION REQUESTED: Reviews for end-use product characterization and waiver requests for toxicity, pathogenicity and infectivity testing.

CONCLUSION: Product Characterization – **SUPPLEMENTAL but Upgradeable** - Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; a revised CSF should be provided listing endotoxin as a contaminant, nominal concentrations of all inert ingredients, minimum PFU/ml for each monophage in ECP-100™ (consistent with the manufacturing process), removing the word 'specific' from the active ingredient description or provide supporting tests for review; a label matching the revised CSF should be provided with clear usage and dilution rates listed. Waiver requests for: Acute Oral Toxicity / Pathogenicity; Acute Pulmonary Toxicity / Pathogenicity; Acute Injection Toxicity / Pathogenicity; Cell Culture; Acute Oral Toxicity; Acute Dermal Toxicity; Acute Inhalation Toxicity; Acute Eye Irritation; Acute Dermal Irritation; and Temporary Food Tolerance Exemption – **SUPPLEMENTAL but Upgradeable** - Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided; a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register.

DATA REVIEW RECORD:

Active Ingredient:	Lytic monophages specific for *E. coli* O157:H7.
Product Name:	ECP-100™.
Company Name:	Intralytix, Inc.
EPA Reg. No.:	74234-EUP-E.
Chemical Number:	Not yet assigned.
Decision Number:	416027.
DP Barcode:	369677.
MRID Nos.:	477868-01; 477868-02; 477868-03.

REVIEW SUMMARY:

Study Type: Product Identity (OPPTS 885.1100)
Manufacturing Process (OPPTS 885.1200)
Discussion of Formation of Unintentional Ingredients (OPPTS 885.1300)
Analysis of Samples (OPPTS 885.1400)
Certification of Limits (OPPTS 8850.1500)
Enforcement Analytical Method (OPPTS 830.1800)
Physical and Chemical Characteristics (OPPTS 830.6302-830.7300).

MRID Nos.: 477868-01; 477868-02.

Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.

Study Summary: Three monophage active against a wide range of *E. coli* O157:H7 strains are produced in *E. coli* hosts which lyse as the phage matures [redacted]

Classification: SUPPLEMENTAL but Upgradeable - ***For the EUP:*** Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; a revised CSF should be provided listing endotoxin as a contaminant, nominal concentrations of all inert ingredients, minimum PFU/ml for each monophage in ECP-100™ (consistent with the manufacturing process), removing the word 'specific' from the active ingredient description or provide supporting tests for review; a label matching the revised CSF should be provided with clear usage and dilution rates listed. ***For registration;*** Methodology (including reagents and protocols) for the PFGE, RFLP and amplicon identity tests should be submitted, information on toxicology for Gram-negative endotoxin, or product toxicology testing, to support the proposed endotoxin limit should be submitted and proposed endotoxin limits should be standardized in the manufacturing process and on the CSF; storage stability must be addressed by data and accompanied by a 'use-by' date on the label; an additional two batch analyses should be submitted.

Study Type: Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acute Dermal Irritation (OPPTS 870.2500).

MRID Nos.: 477868-03.

Test Material: ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.

Study Summary: Bacteriophages are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered

ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP.
Classification: SUPPLEMENTAL but Upgradeable - *For the EUP:* Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided.

Study Type: Temporary Food Tolerance Exemption.
Test Material: Lytic monophages for *Escherichia coli* O157:H7; ECML-4, ECML-117 and ECML-134.
Study Summary: Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use

rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces.
Classification: SUPPLEMENTAL but Upgradeable - *For the EUP:* Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided; a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register.

*** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION ***

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Product Identity (OPPTS 885.1100); Manufacturing Process (OPPTS 885.1200); Discussion of Formation of Unintentional Ingredients (OPPTS 885.1300); Analysis of Samples (OPPTS 885.1400); Certification of Limits (OPPTS 885.1500); Enforcement Analytical Method (OPPTS 830.1800); Physical and Chemical Characteristics (OPPTS 830.6302-830.7300).
MRID Nos.	477868-01; 477868-02.
Test Material	ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study No.	None given.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Titles of Reports	ECP-100™ – Product Identity, Manufacturing Process, Sample Deposition and Discussion of the Formation of Impurities; ECP-100™ – Analysis of Samples, Certification of Limits, Physical and Chemical Characteristics.
Author	Eliot Harrison.
Study Completed	May 30, 2009.
Study Summary	Three monophage active against a wide range of *E. coli* O157:H7 strains are produced in *E. coli* hosts which lyse as the phage matures [redacted][10]
Classification	**SUPPLEMENTAL** but **Upgradeable** - ***For the EUP:*** Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; a revised CSF should be provided listing endotoxin as a contaminant, nominal concentrations of all inert ingredients, minimum PFU/ml for each monophage in ECP-100™ (consistent with the manufacturing process), removing the word 'specific' from the active ingredient description or provide supporting tests for review; a label matching the revised CSF should be provided with clear usage and dilution rates listed. ***For registration;*** Methodology (including reagents and protocols) for the PFGE, RFLP and amplicon identity tests should be submitted, information on toxicology for Gram-negative endotoxin, or product toxicology testing, to support the proposed endotoxin limit should be submitted and proposed endotoxin limits should be standardized in the manufacturing process and on the CSF; storage stability must be addressed by data and accompanied by a 'use-by' date on the label; an additional two batch analyses should be submitted.
Good Laboratory Practice	A signed and dated (May 30, 2009) GLP statement was provided; These studies are not subject to the requirements of 40 CFR Part 160.

I. PRODUCT IDENTITY: Product Name: ECP-100™.

Taxonomy and history of strain:

i) Taxonomic designation: Lytic monophages ECML-4, ECML-117 and ECML-134. Sequencing revealed several hundred open reading frames (Table 1) for which the majority are putative proteins.
ii) Original isolates: ECML-134 was isolated from a product commercialized in the Republic of Georgia (i.e. Pyophage) 3/8/2005 while ECML-4 (11/18/2004) and ECML-117 (12/21/2004) were isolated from water obtained at the Inner Harbor, Baltimore, MD. Monophage are collected as plaques in an *E. coli* lawn overlay plate, then serially plated until they yield a single band by PFGE and stable patterns from RFLP (DNA) and SDS-PAGE (protein), and homogenous morphology by

electron microscopy. Full geonome sequences were supplied, as were lytic profiles for monophage against a bacterial (*E. coli* O157:H7, *E. coli* non-O157:H7 and non-*E. coli*) library.

iii) Origin and Natural occurrence: The origin of the Pyophage isolate (a commercial isolate from the Republic of Georgia) is unknown, while the ECML-4 and ECML-117 occur in surface waters leading to the Chesapeake Bay, MD. Monophage were categorized in the Myoviridae phage family (Ackerman and Berthiaume 1995).

iv) Strain preservation and maintenance during product development: Monophage working stocks are stored at 2-6°C in phosphate buffered saline kept in the dark. Monophage seed stocks are freeze-dried in 5% sodium glutamate / 0.5 % gelatin and sealed into glass ampoules. Monophage are deposited to ATCC as PTA-7948 (ECML-4), PTA-7950 (ECML-117) and PTA-7949 (ECML-134).

v) Morphological and physiological characteristics: See table 1.

Table 1. Morphological and physiological properties reported for individual monophage:

Monophage	Head (nm)	Tail (nm)	Monophage Length (nm)	PFGE bands	RFLP Fragments[1]	Size (BP)[2]	GC%[3]	# ORF[4]
ECML-4	82	123	205	1	17	157,308	45	202
ECML-117	79	117	196	1	5	66,854	46	103
ECML-134	114	106	220	1	18	166,783	35	157

[1] ECML-134 and ECML-117 / EcoRV digest; ECML-4 AflIII digest; [2] Sum of all fragments; [3] Guanine + Cytosine content; [4] Open reading frames present.

vi) Host range analysis:

a) Pest host range: The product marketed in the Republic or Georgia, Pyophage (where ECML-134 was isolated) contains five phage isolated from *Staphylococcus aureus*, *Proteus vulgaris / mirabilis*, *Streptococcus*, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from "prurulent infections" and active against these bacteria with a claimed success rate of 90-95%. It is likely that the monophage can lyse other strains of *E. coli* than just O157:H7, though this was not reported.

b) Lytic profiles: Reported lysis testing here was for *E. coli* O157:H7 specifically (MRID 477868-01. pgs. 12-17 of 20) though page 5 of 20 MRID 477868-01 also reports testing non-O157:H7 *E. coli* and bacteria other than *E. coli* (data not shown). Of 111 O157:H7 strains tested, 11 were not lysed by any of these monophage. ECML-4 lysed 70%, ECML-117 lysed 87% and ECML-134 lysed 65% of the 111 *E. coli* O157:H7 strains tested, respectively. In total, the three monophage lysed 90% of tested *E. coli* O157:H7 strains.

vii) History of use: Pyophage (which contains ECML-134) is marketed in the Republic of Georgia (and possibly elsewhere) for infections, wounds and surgical sites with no contraindications. ECML-4 and ECML-117 are not yet commercialized.

Deficiencies: Full reported host range lysis testing should be submitted for registration.

II. MANUFACTURING PROCESS:

[redacted]

Manufacturing process information may be entitled to confidential treatment

[redacted]

III. Analysis of Samples:
Potency Estimation and Product Viability: Potency must be 0.95-1.5x10^{10} PFU or further concentration or dilution is performed.

Active Ingredient/MPCA: PFGE and RFLP differentiate the monophage and an amplicon identity test shows components in ECP-100™.
Analysis for Microbial Contaminants: Bacterial sterility test – 1 mL phage lysate is plated to Luria Bertani agar 37 ± 2°C 48 ± 4 hr., another 1 mL is added to 9 mL Luria Bertani broth, incubated 37 ± 2°C 24 ± 4 hr. then plated to Luria Bertani agar incubated 37 ± 2°C 24 ± 4 hr. Any growth necessitates another filtration and re-testing.

Table 3. Batch analysis for Monophages:

Monophage	Lot #	PFU/mL[1]	PFGE Bands	RFLP Match	Bacterial Sterility
ECML-4	110306	$4x10^{10}$	1	YES	No Growth
	121506	$2x10^{10}$	1	YES	No Growth
	122106	$2x10^{10}$	1	YES	No Growth
ECML-117	110206	$1x10^{11}$	1	YES	No Growth
	121406	$5x10^{10}$	1	YES	No Growth
	122006	$3x10^{10}$	1	YES	No Growth
ECML-134	102706	$2x10^{10}$	1	YES	No Growth
	121806	$2x10^{10}$	1	YES	No Growth
	122206	$1x10^{10}$	1	YES	No Growth

[1] Minimum $0.95\text{-}1.5x10^{10}$ PFU/mL for each monophage.

Table 4. Batch analysis for ECP-100™:

ECP-100™ Lot #	PFU/mL [1]	Endotoxin [2]	Amplicon Match	Bacterial Sterility
0706L270107	$1x10^{10}$	148,960	YES	No Growth
0706L270213	$1x10^{10}$	148,960	YES	No Growth
0706L270332	$1x10^{10}$	217,528	YES	No Growth

[1] Minimum $0.95\text{-}1.5x10^{10}$ PFU/mL for each monophage; [2] EU/mL – proposed limit here is ≤500,000.

Deficiencies: An additional two batch analyses should be submitted for registration.

IV. DISCUSSION OF FORMATION OF UNINTENTIONAL INGREDIENTS:

In response to an EPA inquiry, the registrant responded with further information on the host bacteria; the strains utilized for culturing phage are claimed to lack shiga toxin production capability, though references obtained for host Ec211/ ECOR-56 / ATCC 35375 were not conclusive. ECP-100 is screened for endotoxin after lysis of cell cultures used to grow the monophage. The CAS number for *E. coli* endotoxin is 67924-63-4. A 3-batch analysis found a range of $1.49\text{-}2.18x10^5$ EU/mL and Intralytix proposes an upper certified limit of $2.5x10^5$ EU/mL (Endotoxin units per mL from a colorimetric Limulus Amebocyte Lysate test; QCL-1000 from Bio-Whittaker) in ECP-100. According to one source 1 EU equals approximately 0.1 to 0.2 ng endotoxin/mL of solution ($2.5x10^5$ EU/mL = ~25,000 ng/mL). FDA limits on endotoxin in sterile water are 0.25 EU/mL for injection and 0.5 EU/mL for inhalation (0.025-0.05 ng/mL). The threshold pyrogenic IV dose (≥ 1.0°F rise in 50% of volunteers) was reported in one study as approximately 4.1 EU/Kg (0.41 ng/Kg) and the FDA pyrogen limit is 5.0 EU/Kg. The registrant cited literature (Leenstra et al. 1996) where oral rinsates in test subjects absent Gram-negative culturable bacteria yielded 3-30 ng/mL endotoxin by the Limulus method (Whittaker). Endotoxin recovered was adjusted 100x for rinsate dilution and a further 1000x based on the assumption that endotoxin was from anaerobic Gram-positive bacteria, resulting in an estimated 0.1-1 mg/mL in saliva due to a claimed lack of sensitivity of the Limulus assay to anaerobic endotoxin. FDA does not discuss lack of sensitivity of the Limulus test in their guidance and the cited reference does not provide further proof beyond discussion in the paper that the Limulus test is not equally sensitive to endotoxin from anaerobes or Gram-positive bacteria; in their assays *Escherichia coli* endotoxin was used to generate the standard curves. Instructions for the Limulus assay cited by the registrant state the assay is not for clinical use: the assay and instructions-for-use utilized by the cited Leenstra et al. paper are not specifically stated.

Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. A review of testing methods for foods and feed currently maintained by FDA and USDA-FSIS indicate that in some cases ECP-100 may cause false-negative results for *E. coli* O157:H7 presence in preliminary (non-culture) test steps. Additionally, effective use of ECP-100 causing lysis of *E. coli* present on food contact surfaces may lead to additional false-negative results with some test preliminary or presumptive methods.

References:

- Leenstra, T.S., J.J.M. van Saene, H.K.F. van Saene and M.V. Martin. 1996. Oral endotoxin in healthy adults. Oral Surgery, Oral Medicine, Oral Pathology 82(6): 637-642.
- FDA BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria. September 2002. Bacteriological Analytical Manual. Chapter 4. Enumeration of *Escherichia coli* and the Coliform Bacteri
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm064948.htm
- USDA-FSIS Microbiology Laboratory Guidebook. Chapter 5.
http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp

Deficiencies: Data or peer-reviewed references were not provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins. Information on toxicological findings for Gram-neagtive endotoxin from peer-reviewed research showing various tested routes of administration, or product toxicology testing, to support the proposed endotoxin limit should be submitted and compared to; a) levels in ECP-100; and b) levels expected in foods from ECP-100 label use rates. Proposed endotoxin limits in MRID 477868-01 (≤250,000 EU/mL) and MRID 477868-02 (≤500,000 EU/mL) should be standardized. Endotoxin should be listed as an impurity on the CSF. A revised CSF and label are required for the EUP.

V. CERTIFICATION OF LIMITS: Table 5 lists the nominal concentration and certified limits for the ingredients in ECP-100™.

TABLE 5. Nominal CSF concentrations and certified limits for ECP-100™ a

Ingredients (CAS number)	PC Code	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
ECP-100 is a mixture of three (3) lytic monophages specific for *E. coli* O157:H7		TGAI	0.00027	0.00024	0.00030
Inert Ingredients					
[redacted]					

[a]Data from CSF (5/30/2009) and MSDS (email from Eliot Harrison 12/1/2009).

Deficiencies: Data submitted does not support the statement that monophage are 'specific' for *E. coli* O157:H7 though they are 'active' or 'lytic' against the *E. coli* O157:H7 strains tested; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided. [redacted] is listed in the MSDS but not listed on the CSF, and nominal concentrations for components of PBS are not listed on the CSF or in the MSDS provided. The CSF should contain ingredient levels as formulated into ECP-100 (the MSDS shows a 10x solution). Minimum PFU/mL for each monophage in ECP-100™ should be listed separately on the CSF and identically on the label. Label use rates specify dilution to 10^9

PFU/mL though there is no specific method to perform this dilution and determine combined monphage PFU/mL in working solutions stated in instructions on the label. The CSF and label should be updated for the EUP.

VI. ENFORCEMENT ANALYTICAL METHOD: Not required.

Deficiencies: None.

VII. PHYSICAL AND CHEMICAL CHARACTERISTICS:

TABLE 6. Physical and Chemical Properties for ECP-100™ [a]

Guideline Reference No./Property	Description of Result
830.6302 Color	Clear to opalescent.
830.6303 Physical State	Liquid.
830.6304 Odor	Odorless
830.6313 Stability	Not applicable due to storage at 2-6°C in a non-metallic container.
830.6314 Oxidation/Reduction: Chemical Incompatibility	N/A, the product does not contain any oxidizing or reducing agents.
830.6315 Flammability	N/A, the product does not contain combustible liquids.
830.6316 Explodability	N/A, the product does not contain potentially explosive ingredients.
830.6317 Storage Stability	Not addressed.
830.6319 Miscibility	N/A, the product is not diluted with petroleum solvents.
830.6320 Corrosion Characteristics	Packaged in plastic; product is >99% PBS and not expected to cause corrosion.
830.6321 Dielectric Breakdown Voltage	N/A, the product is not for use around electrical equipment.
830.7000 pH	7.2-7.3.
830.7050 UV/Visible Absorption	Not required for EP.
830.7100 Viscosity	Essentially that of water (product is >99% PBS).
830.7200 Melting Range	Not required for EP.
830.7220 Boiling Range	Not required for EP.
830.7300 Bulk Density	1.007-1.008 g/mL; approximately 8.33 Lbs./gal.
830.7370 Dissociation Constant in Water	Not required for EP.
830.7550 Partition Coefficient	Not required for EP.
830.7840 Water Solubility	Not required for EP.
830.7950 Vapor Pressure	Not required for EP.

[a] Data from MRID 477868-02.

Deficiencies: Storage stability must be addressed by data and accompanied by a 'use-by' date on the label.

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.
EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Waiver requests for: Acute Oral Toxicity / Pathogenicity (OPPTS 885.3050); Acute Pulmonary Toxicity / Pathogenicity (OPPTS 885.3150); Acute Injection Toxicity / Pathogenicity (OPPTS 885.3200); Cell Culture (OPPTS 885.3500); Acute Oral Toxicity (OPPTS 870.1100); Acute Dermal Toxicity (OPPTS 870.1200); Acute Inhalation Toxicity (OPPTS 870.1300); Acute Eye Irritation (OPPTS 870.2400); Acut Dermal Irritation (OPPTS 870.2500).
MRID Nos.	477868-03.
Test Material	ECP-100™ containing lytic monophages specific for *E. coli* O157:H7.
Study No.	ECP-100/ SA001.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Waiver Requests for Microbial Pesticide Toxicology Data Requirements and Discussion of Safety Issue
Author	Eliot Harrison.
Study Completed	May 30, 2009.
Study Summary	Bacteriophages are present in high numbers in the environment, including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage are viruses that only infect specific bacteria. Bacteriophage presence reported in foods and feeds ranges from 10^{1}-10^{5} PFU/100 g meats and up to 10^{7} PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. A literature review of the >80 year history of therapeutic bacteriophage use in Eastern Europe and the former Soviet Union, and mostly 'pre-antibiotic age' usage in Western countries, shows there have been no adverse effects reported from widespread use, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^{9}$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^{3}-10^{5} PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Reporting of any hypersensitivity incidents related to use of ECP-100 or individual monophage is required for the EUP.
Classification	**SUPPLEMENTAL but Upgradeable** - ***For the EUP:*** Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided.
Good Laboratory Practice	A signed and dated (May 30, 2009) GLP statement was provided; These studies either were not subject t the requirements of 40 CFR Part 160, the requirements were not met or the submitter does not know if GLP was followed for data collection.

The registrant included a thorough literature review and set of rationale to waive requirements for toxicology, pathogenicity, infectivity and irritation testing for the component monophage. In

addition, MSDS for inert ingredients, and their status as minimal risk were submitted by email. Since this is both a manufacturing-use and end-use product without registered TGAIs, there is only one set of data waivers submitted.

RATIONALE:
Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English work, and in a search of Western/English language literature for any reported adverse effects, in a few cases reporting controlled scientific studies. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment, including in non-polluted and treated drinking water, and in foods and feeds, without any known harmful effects.

Inert Ingredients: Phopshate buffered saline composed of [redacted] In essence the Phosphate Buffered Saline represents a solution at physiological pH and osmolarity that is not expected to have any harmful effects from contact with eyes or skin, nor from ingestion or inhalation.

Presence in the Environment:
According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5\text{x}10^8$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained $10^3\text{-}10^5$ PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

- Ashelford, K.E., Day, M.J. and Fry, J.C. 2003. Elevated Abundance of Bacteriophage Infecting Bacteria in Soil. Appl. Environ. Microbiol. 69, 285-289.
- Bergh, O., Børsheim, K.Y., Bratbak, G. & Heldal, M. 1989. High abundance of viruses found in aquatic environments. Nature 340, 467–468.
- Boehme, J., Frisher, M.E., Jiang, S.C., Kellogg, C.A., Pichard, S., Rose, J.B., Steinway, C. and Paul, J.H. 1993. Viruses, bacterioplankton, and phytoplankton in the southeastern Gulf of Mexico: distribution and contribution to oceanic DNA pools. Ma. Ecol. Prog. Ser. 97, 1–10.
- Børsheim, K.Y. 1993. Native marine bacteriophages. FEMS Microbiol. Ecol. 102, 141–159.
- Calci, K.R., Burkhardt III, W., Watkins, W.D. & Rippey, S.R. 1998. Occurrence of Male-Specific Bacteriophage in Feral and Domestic Animal Wastes, Human Feces, and Human Associated Wastewaters. Appl. Environ. Microbiol. 64, 5027-5029.
- Cochlan, W.P., Wikner, J., Steward, G.F., Smith, D.C. & Azam, F. 1993. Spatial distribution of viruses, bacteria and chlorophyll a in neritic, oceanic and estuarine environments. Mar. Ecol. Prog. Ser. 92, 77–87.
- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
- Fuhrman, J.A. 1999. Marine viruses and their biogeochemical and ecological effects. Nature 399, 541-548.
- Maranger, R., Bird, D. F. & Juniper, S. K. 1994. Viral and bacterial dynamics in arctic sea ice during the spring algal bloom near Resolute, NWT, Canada. Mar. Ecol. Prog. Ser. 111, 121–127.
- Maranger, R. & Bird, D.E. 1996. High concentrations of viruses in the sediments of Lac Gilbert, Quebec. Microb. Ecol. 31, 141–151.
- Noble, R.T. & Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14, 113–118.
- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.
- Proctor, L.M. & Fuhrman, J.A. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343, 60–62.
- Sieburth, J.M., Johnson, P.W. & Hargraves, P.E. 1988. Ultrastructure and ecology of *Aureococcus anophagefferens* gen. et sp. nov. (Chrysophyseae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, Summer 1985. J. Phycol. 24, 416–425.
- Steward, G.F., Smith, D.C. & Azam, F. 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Sea. Mar. Ecol. Prog. Ser. 131, 287–300.
- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophages are commonly consumed by humans via various foods. In this context, bacteriophages have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the

ground beef and chicken meat sold at retail contain various levels of various bacteriophages. For example, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from 3.3-4.4x10^{10} PFU/100 g of fresh chicken, up to 3.5x10^{10} PFU/100 g of fresh pork, and up to 2.7x10^{10} PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of 10^1-10^5 PFU/100 g meats and up to 10^5 PFU/g (10^7 PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

- Ackermann, H. W. 1997. Bacteriophage ecology. Pages 335-339 in: Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez, eds. Brazilian Society for Microbiology. Quoted in: http://www.apsnet.org/online/feature/phages/
- Armon, R., Araujo, R., Kott, Y., Lucena, F. and Jofre, J. 1997. Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. J. Appl. Microbiol. 83, 627-633.
- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. 2003. Isolation and Characterization of *Campylobacter* Bacteriophages from Retail Poultry. Appl. Environ. Microbiol. 69, 4511-4518.
- Gautier, M., Rouault, A., Sommer, P. and Briandet, R. 1995. Occurrence of *Propionibacterium freudenreichii* Bacteriophages in Swiss Cheese. Appl. Environ. Microbiol. 61, 2572-2576.
- Greer, G.G. 2005. Bacteriophage Control of Foodborne Bacteria. J. Food Prot. 68, 1102-1111.
- Kennedy Jr., J.E., Oblinger, J.L. and Bitton, G. 1984. Recovery of Coliphages from Chicken, Pork Sausage and Delicatessen Meats. J. Food Prot. 47, 623-626.
- Kennedy Jr., J.E., Wei, C.I. and Oblinger, J.L. 1986. Methodology for Enumeration of Coliphages in Foods. Appl. Environ. Microbiol. 51, 956-962.
- Maciorowski, K.G., Pillai, S.D. and Ricke, S.C. 2001. Presence of Bacteriophages in Animal feed as Indicators of fecal Contamination. J. Enciron. Sci. Health B36, 699-708.
- Whitman, P.A. and Marshall, R.T. 1971. Isolation of Psychrophilic Bacteriophage-Host Systems from Refrigerated Food Products. Appl. Microbiol. 22, 220-223.

Health Effects:

Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects (Alisky et al 1998, Sulakvelidze et al 2001). Immune system clearance of bacteriophage at

various stages of immune impairment without adverse effects from the bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight (Lopez 1975, Ochs et al. 1992). *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication (Bruttin and Brussow 2005).

References:

-Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. 1998. Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15.
- Bruttin, A. & Brussow, H. 2005. Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878.
- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. & Wedgewood, R.J. 1975. Defective antibody response to bacteriophage ΦX174 in Down syndrome. J. Pediatrics 86, 207-211.
- Ochs, H.D., Buckley, R.H., Kobayashi, R.H., Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. & Herchfeld, M.S. 1992. Antibody Responses to Bacteriophage ΦX174 in Patients With Adenosine Deaminase Deficiency. Blood 80, 1163-1171.
-Sulakvelidze, A., Alavidze, Z. & Morris Jr., J.G. 2001. Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659.

Transduction, Lysogeny and Bacteriophage Sequencing:

The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:

- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Deficiencies: Data or peer-reviewed references were not provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 is serotype 06:H1 and does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided.

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.

EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Temporary Food Tolerance Exemption Petition.
MRID Nos.	None.
Test Material	Lytic monophages for *Escherichia coli* O157:H7; ECML-4, ECML-117 and ECML-134.
Study No.	None given.
Sponsor	Intralytix, Inc.; 701 E. Pratt St.; Baltimore, MD 21202.
Testing Facility	None.
Titles of Reports	Petition Requesting a Temporary Tolerance Exemption from the Requirement of a Tolerance for *E. coli* O157:H7 Specific Bacteriophages used on Food-Contact Surfaces in Food Processing Plants.
Author	None given.
Study Completed	None given.
Study Summary	Literature submitted established that bacteriophage have been used historically and through modern times in lieu of, or to assist the action of antibiotics. Bacteriophage are viruses that only infect specific bacteria. Clinical uses encompass all manner of administration from injection/I.V. and surgical wound applications to topical and ingestible preparations and to test normal and variously impaired human immune system function. There have been no reports of adverse effects from such administrations in literature mostly reviewing non-English language work, and in a search of Western/English language literature for any reported adverse effects, in a few cases using controlled scientific studies. Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight. *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication. Also submitted were literature citations showing that bacteriophage are present in high numbers in the environment including in non-polluted waters up to 10^{10} PFU/L and in treated drinking water. Bacteriophage presence reported in foods and feeds ranges from 10^1-10^5 PFU/100 g meats and up to 10^7 PFU/100 g in cheese without any known harmful effects. Bacteriophages are common and abundant in soils and in a wide range of plant materials. The main risk issue associated with use of bacteriophage as an antimicrobial agent is to ensure use of bacteriophage and host bacteria lacking toxin production or pathogenicity factors. Cell-free filtrates are utilized for the pesticidal product and analysis of the host strains and bacteriophage properties show one of the host strains is atoxigenic, and bacteriophage sequences did not reveal any known toxin genes, specifically those associated with *E. coli*, including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Bacteriophage combined in ECP-100 are 0.00027% by weight and label use rates are a 10^9 PFU/mL working solution applied to food and non-food contact surfaces.
Classification	**SUPPLEMENTAL but Upgradeable** - ***For the EUP:*** Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided; a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register.
Good Laboratory Practice	Not applicable.

Presence in the Environment:

According to one review (Fuhrman 1999) "The first reports of high viral abundance, exceeding the typical bacterial abundance of 10^9 per litre (Sieburth et al. 1988, Bergh et al. 1989, Proctor and Fuhrman 1990, Wommack et al. 1992), awakened interest in this topic. Many subsequent studies (Wommack et al. 1992, Børsheim 1993, Cochlan et al. 1993, Paul et al. 1993, Boehme et al. 1993, Maranger et al. 1994, Hara et al. 1996, Maranger & Bird 1996, Steward et al. 1996, Noble & Fuhrman 1998) have shown that viruses are consistently the most abundant biological entities in the sea—nearshore and offshore, tropical to polar, sea surface to sea floor, and in sea ice and sediment pore water. Viral abundances are typically 10^{10} per litre in surface waters (about 5–25 times the bacterial abundance), and follow the same general abundance patterns as bacteria. These patterns include a decrease of about one order of magnitude between rich coastal waters and oligotrophic (nutrient poor) open ocean, a decrease of between five- and tenfold from the euphotic zone to the upper midwaters (for example, 500 m depth), and a further decrease several-fold to abyssal depths. As occurs with bacteria, sea ice is highly enriched in viruses compared with the water beneath it (Maranger et al. 1994), and sediment pore waters are highly enriched compared with overlying water (Paul et al. 1993, Steward et al. 1996)." In soil, bacteriophage were "at least 350-fold more than the highest numbers estimated from traditional viable plaque counts" or in the range of $0.15\text{-}1.5 \times 10^8$ PFU/g soil (Ashelford et al. 2003). Sewage plant effluents contained $10^3\text{-}10^5$ PFU/100 mL sewage with an approximate decrease of 10^1 PFU/100 mL with treatment (Calci et al. 1998).

References:

- Ashelford, K.E., Day, M.J. and Fry, J.C. 2003. Elevated Abundance of Bacteriophage Infecting Bacteria in Soil. Appl. Environ. Microbiol. 69, 285-289.
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- Hara, S., Koike, I., Terauchi, K., Kamiya, H. & Tanoue, E. 1996. Abundance of viruses in deep oceanic waters. Mar. Ecol. Prog. Ser. 145, 269–277.
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- Paul, J.H., Rose, J.B., Jiang, S.C., Kellogg, C.A. & Dickson, L. 1993. Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl. Environ. Microbiol. 59, 718–724.

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- Wommack, K.E., Hill, R.T., Kessel, M., Russek-Cohen, E. & Colwell, R.R. 1992. Distribution of viruses in the Chesapeake Bay. Appl. Environ. Microbiol. 58, 2965–2970.

Presence in Foods:

According to one source (Ackerman 1997) bacteriophage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors... healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat." The registrant submitted a literature review stating "Bacteriophages are commonly consumed by humans via various foods. In this context, bacteriophages have been commonly isolated from a wide range of food products, including ground beef, pork sausage, chicken, farmed freshwater fish, common carp and marine fish, oil sardine, raw skim milk, and cheese (Atterbury et al. 2003, Gautier et al. 2005, Greer 2005, Kennedy et al. 1986, Kennedy et al. 1984, Whitman & Marshall 1971). Several studies have suggested that 100% of the ground beef and chicken meat sold at retail contain various levels of various bacteriophages. For example, bacteriophages were recovered from 100% of examined fresh chicken and pork sausage samples and from 33% of delicatessen meat samples analyzed (Kennedy et al. 1984). The levels ranged from 3.3-4.4x10^{10} PFU/100 g of fresh chicken, up to 3.5x10^{10} PFU/100 g of fresh pork, and up to 2.7x10^{10} PFU/100 g of roast turkey breast samples. In another study (Kennedy et al. 1986) samples of fresh chicken breasts, fresh ground beef, fresh pork sausage, canned corned beef, and frozen mixed vegetables were examined for the presence of coliphages. Although only three ATCC strains of *E. coli* were used as indicator host strains, coliphages were found in 48 to 100% of the various food samples examined." Reviewer's note: Indigenous bacteriophage recovered from foods in the cited references were more typically in the range of 10^1-10^5 PFU/100 g meats and up to 10^5 PFU/g (10^7 PFU/100 g) in cheese and PFU numbers depended largely on extraction technique and the choice of host cells for plaque assays. Bacteriophage specific for mammalian fecal bacteria have been detected (presence/absence) in up to 10% of disinfected surface and groundwater water sources in Spain and Israel (Armon et al. 1997). Animal feeds and ingredients were assayed for bacteriophage specific to *Salmonella* and *E. coli* with the result that regardless of storage conditions enrichment led to positive bacteriophage results in all tested materials, and in the majority of replicates (Maciorowski et al. 2001).

References:

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Health Effects:

Much of the >80 year history of therapeutic bacteriophage use was in Eastern Europe and the former Soviet Union, though Western countries used them variously prior to widespread antibiotics usage. Bacteriophage are viruses that only infect select bacterial hosts. Reviews submitted of the examinable literature, much of it in Russian or other non-English language formats, shows there have been no adverse effects reported from widespread use, and in a few cases controlled scientific studies have also shown various benefits without adverse effects (Alisky et al 1998, Sulakvelidze et al 2001). Immune system clearance of bacteriophage at various stages of immune impairment without adverse effects from the bacteriophage was shown in two published studies where humans were administered ΦX174 I.V. at $2x10^9$ PFU/Kg body weight (Lopez 1975, Ochs et al. 1992). *Escherichia coli* bacteriophage T4 administered to healthy human volunteers at 10^3-10^5 PFU/mL in drinking water resulted in detection in feces relevant to dose level, no detection in serum and no decrease in fecal *E. coli* or noticeable bacteriophage replication (Bruttin and Brussow 2005).

References:

-Alisky, J., Iczkowski, K., Rapoport, A. and Troitsky, N. 1998. Bacteriophages Show Promise as Antimicrobial Agents. J. Infection 36, 5-15.
- Bruttin, A. & Brussow, H. 2005. Human Volunteers Receiving *Escherichia coli* Phage T4 Orally: a Safety Test of Phage Therapy. Antimicrob. Agents and Chemo. 49, 2874-2878.
- Lopez, V., Ochs, H.D., Thuline, H.C., Davis, S.D. & Wedgewood, R.J. 1975. Defective antibody response to bacteriophage ΦX174 in Down syndrome. J. Pediatrics 86, 207-211.
- Ochs, H.D., Buckley, R.H., Kobayashi, R.H., Kobayashi, A.L., Sorensen, R.U., Douglas, S.D., Hamilton, B.L. & Herchfeld, M.S. 1992. Antibody Responses to Bacteriophage ΦX174 in Patients With Adenosine Deaminase Deficiency. Blood 80, 1163-1171.
-Sulakvelidze, A., Alavidze, Z. & Morris Jr., J.G. 2001. Bacteriophage Therapy. Antimicrob. Agents and Chemo. 45, 649-659.

Transduction, Lysogeny and Bacteriophage Sequencing:

The main, if perhaps only risk issue associated with use of bacteriophage as an antimicrobial agent (or for therapeutic applications) is to ensure the selection of bacteriophage and host bacteria that are not associated with toxin production or pathogenicity factors, i.e. pathogenicity islands (Hacker and Kaper 2000). In this case, use of cell free filtrates and analysis of the host strains and bacteriophage properties suffice. Use of host strains that are atoxigenic is key to

absence of toxins, including *E. coli* O157:H7 shigatoxins, in end-use products. Analysis of bacteriophage sequences and lytic patterns is key to selecting bacteriophage that are lytic in nature and that do not carry or horizontally pass host genes. The lytic nature of monophages was tested to ascertain they will not horizontally pass host genes; bacteriophage were selected that either completely lyse or have no activity against hundreds of *E. coli* O157:H7 strains; bacteriophage that incompletely lyse *E. coli* were not selected. Sequence analysis of the monophages in ECP-100 did not reveal any known toxins, specifically those associated with bacteriophage (see pages 11-12 of 231, MRID 477868-03), including shigatoxins. Sequence analysis was also used to search for any bacterial 16s rRNA genes, which may indicate lysogenic phage – none were found in any of the monophage genomes.

References:

- Hacker, J. & J.B. Kaper. 2000. Pathogenicity Islands and the Evolution of Microbes. Annu. Rev. Microbiol. 54, 641-679.

Deficiencies: Data or peer-reviewed references should be provided showing that the host bacterium *E. coli* Ec211/ ECOR-56 / ATCC 35375 does not produce shigatoxins; data from host-range testing of non-O157:H7 *E. coli* and bacteria other than *E. coli* should be provided; a temporary food tolerance exemption petition listing individual monophage should be submitted in a format that can be published in the Federal Register.

PRIA 2 – 21 Day Content Screen Review Worksheet

(EPA/OPP Use Only)

3/23/09

21 Day Screen Start Date: 6-22-09

Experts In-Processing Signature: MF HARRINGTON Date 6-29-09 Fee Paid: Yes ✓

Division management contacted on issues No_____Yes_____Date_____

EPA Reg. Number: 74234-EUP-E	EPA Receipt Date: 6-22-09

	Items for Review	Yes	No	N/A*
1	**Application Form** (EPA Form 8570-1)(link to form) signed & complete including package type			X
2	**Confidential Statement of Formula** all boxes completed, form signed, and dated (EPA Form 8570-4) (Link to form)	X		
	a) All inerts (link to http://www.epa.gov/opprd001/inerts/), including fragrances, approved for the proposed uses (see Footnote A) See comments — yes: X; no:			
3	**Certification with Respect to Citation of Data** (EPA Form 8570-34) (Link to form) completed and signed (N/A if 100% repack)	X		
	Certificate and data matrix consistent	X		
	If applicant is relying on data that are compensable, is the offer to pay statement included. (see Footnote B) — yes: ; no:			
	If applicable, is there a letter of Authorization for exclusive use only.			
4	**Formulator's Exemption Statement** (EPA Form 8570-27) (Link to form) completed and signed (N/A if source is unregistered or applicant owns the technical)			X
5	**Data Matrix** (EPA Form 8570-35) (Link to form) both internal and external copies (PR 98-5) (Link to PR 98-5) completed and signed (N/A if 100% repack)	X		
	a) Selective Method (Fee category experts use) — yes: X; no:			
	b) Cite-All (Fee category experts use) — yes: ; no:			
	c) Applicant owns all data (Fee category experts use) — yes: ; no:			
6	**5 Copies of Label (link to http://www.epa.gov/oppfead1/labeling/lrm/) (Electronic labels on CD are encouraged and guidance is available)(link to** http://www.epa.gov/pesticides/regulating/registering/submissions/index.htm#labels)	X		

7	**Is the data package consistent with PR Notice 86-5** (link to PRN 86-5)	X		
8	**Notice of Filing (link to http://www.epa.gov/pesticides/regulating/tolerance_petitions.htm) included with petitions (link to http://www.epa.gov/pesticides/regulating/tolerances.htm)**			X
9	**If applicable for conventional applications, reduced risk rationale (link to http://www.epa.gov/opprd001/workplan/reducedrisk.html)**			X
10	**Required Data (link to http://www.epa.gov/pesticides/regulating/data_requirements.htm) and/or data waivers. See Footnote C.**			
	a) List study (or studies) not included with application			

202

Comments:

Inerts

[redacted] be entitled to confidential treatment* is found on the list but the CAS # is different than the # listed on the CSF.

Studies passed 86-5 review

477868-01 to 477868-03.

MRID 477868

* N/A – Not Applicable

Footnotes

A. During the 21 day initial content review, all CSFs will be reviewed to determine whether all inerts listed, including fragrances, are approved for the proposed uses. If an unapproved inert is identified, the applicant must either 1) resolve the inert issue by, for example, removing the inert, substituting it with an approved inert, submitting documentation that EPA approved the inert for the proposed pesticidal uses, correcting mistakes on the CSF, etc. or 2) provide the data to support OPP approval of the inert or 3) withdraw the application. Removing or substituting an inert ingredient will require a new CSF and **may** require submission of data. All information, forms, data and documentation resolving the inert issue must have been received by the Agency or the application withdrawn within the 21 day period, otherwise, the Agency will reject the application as described below.

To successfully complete this aspect of the 21 day initial content screen, applicants are **strongly encouraged** to verify that all inert ingredients have been approved for the application's uses **even if a product is currently registered** by consulting the inert Web

site [link to http://www.epa.gov/opprd001/inerts/lists.html] and if the inert is not approved, to **obtain the necessary inert approval prior to submitting an application to register a pesticide product containing that inert ingredient.** Some inert ingredients are no longer approved for food uses or certain types of uses. The name and/or CAS number on a CSF must match the name and CAS number on this web site. Simple typographical errors in the name or CAS number have resulted in processing delays.

If an inert is not listed on the inert ingredient web site and the applicant believes that the inert has been approved, the applicant should contact the Inert Ingredient Assessment Branch (IIAB) at inertsbranch@epa.gov and resolve the issue. Copies of the correspondence with IIAB resolving the issue should accompany the application. All new inerts except PIP inerts are reviewed by IIAB. The IIAB should also be contacted for any questions on what supporting data needs to be submitted for and the Agency's inert review process. Questions on PIP inerts should be directed to the Chief of Microbial Pesticides Branch [Link to http://www.epa.gov/oppbppd1/biopesticides/contacts_bppd.htm].

When a brand, trade, or proprietary name of an inert ingredient is listed on a CSF, additional information such as an alternate name of the inert, CAS number or other information [link to http://www.epa.gov/opprd001/inerts/tips.pdf] must also be included to enable the Agency to determine if it has been approved. Each component of an inert mixture (including a fragrance) must be identified. In some cases, the supplier of the mixture or fragrance may need to provide this information to the Agency. Prior to the Agency's receipt of an application, applicants must arrange with a proprietary mixture or fragrance supplier to provide the component information to the Agency or promptly upon EPA's request. If the inert ingredients in a proprietary blend (including fragrances) cannot or are not identified or provided within the 21-day content review period, the Agency will reject the application.

During the 21 day content review, applicants should submit information to the individual identified by the Agency when the applicant is informed of an unapproved inert.

Unapproved Inerts Identified on CSFs

All applications except conventional new products and PIPs

Once an unapproved inert is identified on a CSF, the Agency will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or

2. Submit the information and data needed for the Agency to approve the unapproved inert. If this option is selected and implemented, the Agency may request an extension in the PRIA decision review timeframe to accommodate the inert review/approval process;

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of these options is selected and implemented by the applicant within the 21 day content review period, the Agency will reject the application and retain 25% of the full fee of the category identified.

Conventional New Product Applications

When the Registration Division identifies an unapproved inert on a CSF with an application for a new product that the applicant has not identified as requiring an inert approval (R311, R312 or R313), it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or

2. Submit the information and data needed for the Agency to approve the unapproved inert, including any required petition to establish or amend a tolerance or exemption from a tolerance. (This option may change the PRIA category for the application, which could require a longer decision review time and a larger fee. If additional fees are due, they must be received by the Agency within the 21 day content review period.)

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21-day content-review period, the Agency will reject the application and retain 25% of the appropriate fee for the new product-inert approval category.

PIP Applications

When the Biopesticide and Pollution Prevention Division identifies an unapproved inert on a PIP CSF and a request to approve the inert does not accompany the application, it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the spelling or name of the inert to that in 40 CFR 174, or providing documentation that the inert has been approved; or

2. Submit the information and data needed for the Agency to approve the unapproved inert. If an inert ingredient tolerance exemption petition is required, the petition must be received by the Agency and the B903 fee paid within the 21 day period. If this option is selected and implemented, the Agency will discuss harmonizing the timeframe for both actions.

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21 day content review period, the Agency will reject the application and retain 25% of the fee.

B. A policy on documentation of offers to pay is still being developed, however, for a me-too or fast track (similar/identical) new product, R300 or A530, an application without the necessary authorizations of offers to pay will be placed into either R301 or A531. The Agency recommends that authorizations of offers to pay be submitted with other PRIA applications to avoid delays in the Agency's decision.

C. Biopesticide applicants are advised to contact the Agency and discuss study waivers prior to submitting their application to the Agency. Documentation of such discussions should be submitted with the study waiver.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

June 29, 2009

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP Decision Number: D-416027
EPA File Symbol or Registration Number: 74234-EUP-E
Product Name: ECP-100 on food and non-food contact surfaces in food-processing plants
EPA Receipt Date: 22-Jun-2009
EPA Company Number: 74234
Company Name: INTRALYTIX, INC.

ELIOT HARRISON
AGENT FOR: INTRALYTIX, INC.
122 C STREET, NW, SUITE 740
WASHINGTON, DC 20001

SUBJECT: Receipt of EUP Application and 75% Small Business Waiver Request

Dear Registrant:

The Office of Pesticide Programs has received your EUP application, 75% small business waiver request, and cerification of payment. If you submitted data with this application, the results of the PRN-86-5 screen will be communicated separately. During the administrative screen, the Office of Pesticide Programs has determined that this Action is subject to a Pesticide Registration Service Fee as defined in the Pesticide Registration Improvement Act.

The Action has been identified as Action Code: A520
NEW USE;EUP;

Your request for waiver has been forwarded for review. You will be notified in writing when a determination is made regarding your request. If your waiver request is approved, the decision review time period will start on the date of approval and we will process a refund of your $1,378 overpayment. If your waiver request is denied, you will receive an invoice for the outstanding balance. If you have any questions, please contact the Pesticide Registration Service Fee Ombudsman at (703) 308-6432.

Sincerely,

Teresa Downs

Front End Processing Staff
Information Technology & Resources Management Division

Fee for Service

{852425$~

This package includes the following

- (•) New Registration
- ( ) Amendment

☑ Studies? ☑ Fee Waiver?

☐ volpay % Reduction: 75

for Division

- (•) AD
- ( ) BPPD
- ( ) RD

Risk Mgr. 31

Receipt No. S- 852425

EPA File Symbol/Reg. No. 74234-EUP-E

Pin-Punch Date: 6/23/2009

☐ This item is NOT subject to FFS action.

Action Code:

Requested: B610

Granted: A520

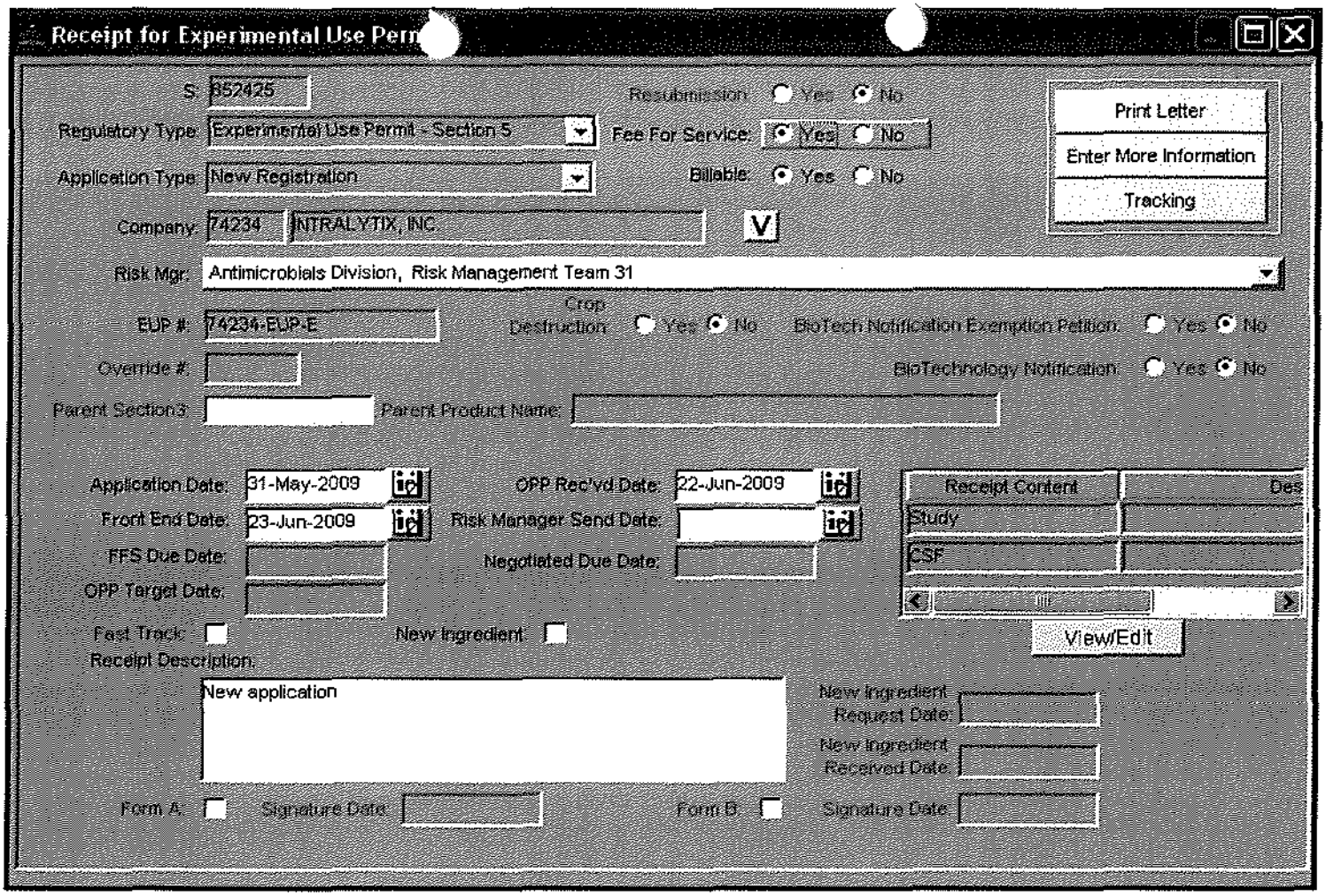
Amount Due: $ 5513.00

Parent/Child Decisions:

☒ Inert Cleared for Intended Use ☒ Uncleared Inert in Product

Reviewer: Team 1 Date: 6-24-09

Remarks:


Receipt for Experimental Use Perm
S: 852425
Resubmission: Yes No
Regulatory Type: Experimental Use Permit - Section 5
Fee For Service: Yes No
Application Type: New Registration
Billable: Yes No
Print Letter
Enter More Information
Tracking
Company: 74234 INTRALYTIX, INC.
Risk Mgr: Antimicrobials Division, Risk Management Team 31
EUP #: 74234-EUP-E
Crop Destruction: Yes No
BioTech Notification Exemption Petition: Yes No
Override #:
BioTechnology Notification: Yes No
Parent Section3:
Parent Product Name:
Application Date: 31-May-2009
OPP Rec'vd Date: 22-Jun-2009
Front End Date: 23-Jun-2009
Risk Manager Send Date:
FFS Due Date:
Negotiated Due Date:
OPP Target Date:
Receipt Content
Des
Study
CSF
Fast Track:
New Ingredient:
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Receipt Description:
New application
New Ingredient Request Date:
New Ingredient Received Date:
Form A:
Signature Date:
Form B:
Signature Date:

experimental program

EXPERIMENTAL PROGRAM FOR ECP-100

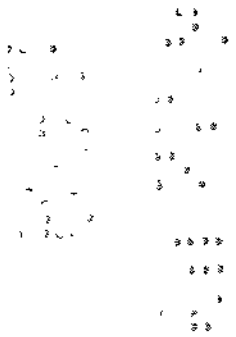


Table 1. Summary of the efficacy data

Matrix	Mean CFU/g		ECP-100 vs. PBS		Significant?
	PBS	ECP-100	Fold reduction	Percent reduction	
Broccoli					
24 ± 4 h	376	2	188-fold	99.5%	Yes
120 ± 4 h	951	9	106-fold	99%	Yes
168 ± 4 h	1329	43	31-fold	97%	Yes
Tomatoes					
24 ± 4 h	117	1	117-fold	99%	Yes
120 ± 4 h	197	12	16-fold	94%	Yes
168 ± 4 h	177	8	22-fold	96%	Yes
Spinach					
24 ± 4 h	1769	0	1769-fold	100%	Yes
120 ± 4 h	2661	11	242-fold	99.6%	Yes
168 ± 4 h	1732	19	91-fold	99%	Yes
Red meat					
24 ± 4 h	1246	67	19-fold	94.5%	Yes

Treatment with ECP-100 (24 ± 4, 120 ± 4 and 168 ± 4 hours, 10°C) significantly reduced ($p < 0.05$) the number of viable E. coli 0157:H7 on fruits, vegetables and red meat contaminated with a mixture of three highly pathogenic strains of the bacterium. The observed reductions ranged from 94% (tomatoes, 120 ± 4 treatment) to 100% (spinach, 24 h). The tomatoes were chosen to represent smooth surfaces, the spinach rough surfaces, and the broccoli and red meat complex surfaces of different characters.

Although bacteriophage have been used extensively as human therapeutics, they have not been previously employed to control environmental pathogens. The purpose of this application for an Experimental Use Permit is to permit assessment of the suitability of bacteriophage as a replacement for the chemical sanitizers currently employed to control *E. coli* 0157:H7 in the beef processing environment that include quaternary ammonium detergents, citric acid, peroxyacetic acid, and sodium hypochlorite.

(II) Location and Size of Trials

Nebraska Washington Kansas Iowa

Trials are planned in beef processing plants, in the states of NE, WA, TX, KA, IA, IL belonging to and operated by Tyson Fresh Meats, Inc. Areas to be included in the trial will be include both food contact and non-food contact surfaces, including floors, walls, areas around drains and gratings, non-food contact equipment as well as tables, conveyors, slicing equipment and related food contact surfaces. Up to 150,000 square feet of interior space will be treated per processing plant.

1. Participants and Cooperators

Study Director
Alexander Sulakvelidze, Ph.D.
Intralytix, Inc.
701 E. Pratt Street
Baltimore, MD 21202

Cooperator
Dean Danilson, Ph.D.
Vice-President, Food Safety and Quality Assurance
Tyson Fresh Meats, Inc.
800 Stevens Port Drive
Dakota Dunes, SD 57409

2. States and Acreages

The trials will be conducted from June 1, 2009 until June 1, 2011 in the states identified in the chart below. The scope of the trials, on an annual basis, is summarized below.

State	Locations Planned	Interior Square Footage Planned	Gallons of Formulation	Pounds of Active Ingredient
NE	2	300,000	3600	0.098
WA	1	150,000	1800	0.049
TX	1	150,000	1800	0.049
KA	2	300,000	3600	0.098
IA	1	150,000	1800	0.049
IL	1	150,000	1800	0.049
Total	8	1,200,000	14,400	0.392

The current plans of Intralytix are to conduct trials at a single Tyson Fresh Meats, Inc. facility in Dakota City, NE. Other sites may be added depending upon the success of the results obtained. However, depending on plant availability, trials may have to be rearranged. Nonetheless, the total number of trials planned, surface treated, gallons of formulation and pounds of active ingredient will remain the same.

3. Program Details

(I) General Description

ECP-100 is a preparation of lytic bacteriophage highly specific for *E. coli* 0157:H7. When ECP-100 bacteriophage encounter *E. coli* 0157:H7, they sequentially attach to the bacterial cell surface, inject their DNA into the bacterium, replicate within the bacterial host, and liberate the phage progeny by lysing the bacterium, rendering it definitively and permanently incapable of causing subsequent food-borne illness. Previous laboratory experiments under controlled conditions have shown that *E. coli* 0157:H7 bacteriophage are capable of achieving substantial reduction of *E. coli* 0157:H7 under experimental conditions.

(III) Applications

All experimental applications will be performed under direct supervision of Intralytix personnel. Intralytix will furnish bacteriophage preparations in sterile 500 ml plastic bottles containing a concentrate of *E. coli* 0157:H7 bacteriophage ECP-100 with a titer of approximately 10^{10} plaque-forming units per ml. At the site of application, the concentrate will be diluted in carboys in either non-chlorinated water or phosphate-buffered saline to a working concentration of 10^9 plaque-forming units per ml. Working *E. coli* 0157:H7 bacteriophage solutions will be applied either by spraying onto surfaces to be treated, or by direct application with a spreading device such as a mop dedicated solely to bacteriophage application. The final bacteriophage concentration on treated surfaces is estimated to be 10^9 to 10^{10} plaque-forming units per square foot at the time of application.

4. Objectives

An Experimental Use Permit will enable Intralytix to determine if the efficacy of *E. coli* 0157:H7 bacteriophage ECP-100 in reducing or eliminating *E. coli* 0157:H7 contamination of surfaces in controlled laboratory experiments can be replicated under field conditions in a working beef processing plant environment. The specific objectives of the EUP program include the determination of the following:

- Effectiveness of ECP-100 in reducing the titer of *E. coli* 0157:H7 with the goal of achieving a minimum of a two-log reduction
- Optimization of application and use under commercial conditions and standards.
- Performance using different modes and schedules of application
- Comparison with existing *E. coli* 0157:H7 control measures
- Suitability of ECP-100 as a replacement for existing control measures for *E. coli* 0157:H7 that presently include chlorine, peroxyacetic acid, quaternary ammonium compounds. Removal of these agents from the beef processing environment is desirable because of hazards posed to workers and the potential for environmental damage.

5. Assessment of Results

Perdue personnel will apply ECP-100 to interior surfaces and non-food contact equipment in accordance with Intralytix protocols. In general, ECP-100 will be applied at a density of 12 ml per square foot once per day. Prior to initiation of treatment, *E. coli* 0157:H7 contamination will be assessed by the routine measures used by Tyson Fresh Meats to test for *E. coli* 0157:H7 species as part of its Hazard Analysis and Critical Control Point (HACCP) program. All microbial assays are performed under the guidelines of both the Association of Official Analytical Chemists and the Microbiology Laboratory Guide of United States Department of Agriculture.

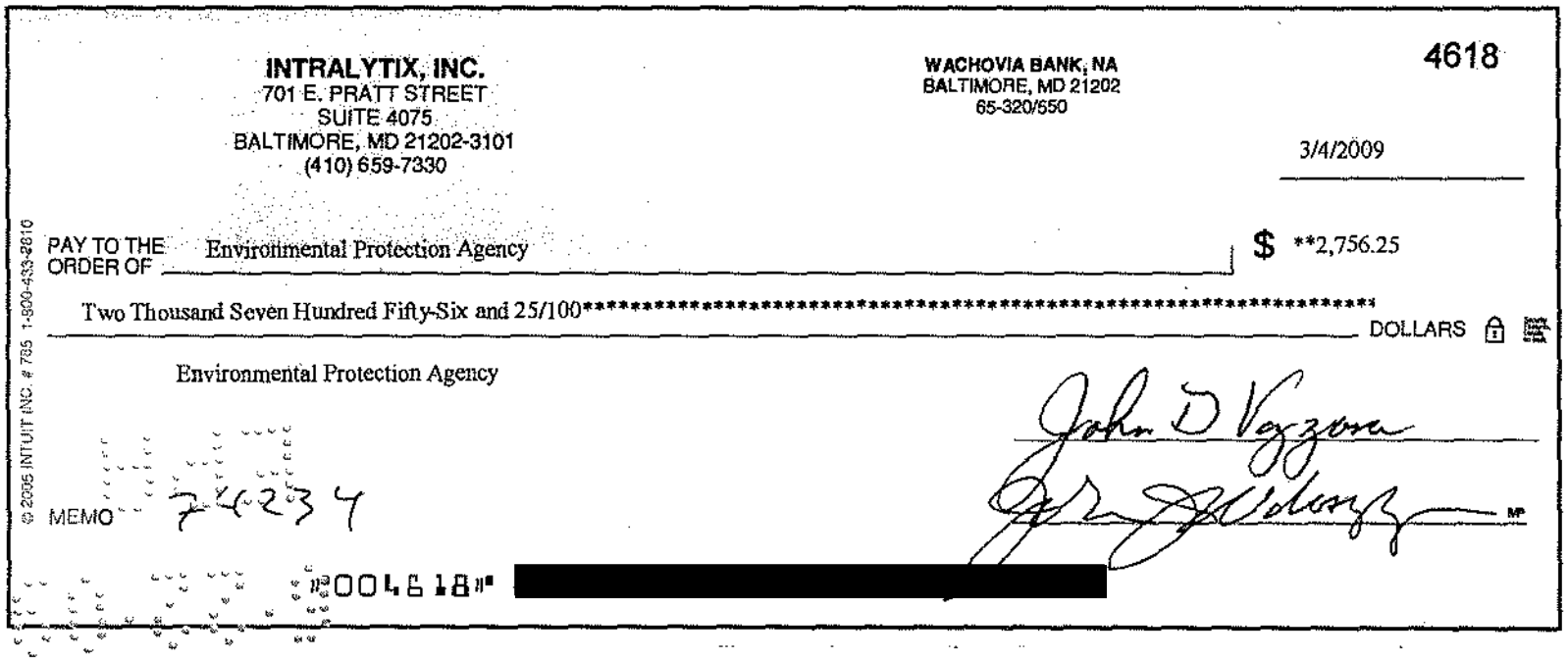
Under the requested EUP, ECP-100 will be evaluated following use of either peroxyacetic acid, quaternary ammonium detergents, or sodium hypochlorite, and will

be tested without use of any of these agents. Rates of *E. coli* 0157:H7 positivity will be monitored by standard Tyson Fresh Meats monitoring procedures. The expectation is that the frequency of positive *E. coli* 0157:H7 cultures will be the same or less than the rate obtained with existing control mechanisms.

6. Project Justification

E. coli 0157:H7 causes significant disease (hemorrhagic diarrhea, hemolytic-uremic syndrome, thrombotic thrombocytopenic purpura) in susceptible individuals, and has been responsible for recalls totalling millions of pound (see http://www.foodsafety.gov/~mow/chap15.html). Current methods of control involve treatment of surfaces in the processing plant with peroxyacetic acid, sodium hypochlorite at 200 ppm, and quaternary ammonium detergents. ECP-100 offers a biological control alternative to these chemical sanitizers.

Commercial/financial information may be entitled to confidential treatment



INTRALYTIX, INC.
701 E. PRATT STREET
SUITE 4075
BALTIMORE, MD 21202-3101
(410) 659-7330

WACHOVIA BANK, NA
BALTIMORE, MD 21202
65-320/550

4618

3/4/2009

PAY TO THE ORDER OF Environmental Protection Agency $ **2,756.25

Two Thousand Seven Hundred Fifty-Six and 25/100********** DOLLARS

Environmental Protection Agency

MEMO 74234

"004618" [redacted]

LEWIS &
HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

May 30, 2009

Velma Noble, Product Manager (31)
Regulatory Management Branch No. 1
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202

re: Application for Experimental Use Permit
Product: ECP-100™
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
PRIA Code: B610 – Food Use; Experimental Use Permit Application, Establish Temporary Tolerance

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am submitting an Experimental Use Permit (EUP) application for the product, ECP-100. This product contains the new active ingredient, *E. coli* 0157:H7 specific bacteriophages, which is a bacterial virus specific against *E. coli* 0157:H7. The objective of the EUP is to evaluate the ability of ECP-100 to control *E. coli* 0157:H7, on both food and non-food contact surfaces, under actual use-conditions.

The following documents are being submitted in support of this EUP:

- Application for Experimental Use Permit Form.
- Confidential Statement of Formula Form (CSF).
- Certification with Respect to Citation of Data Form.
- Data Matrix Chart.
- Proposed Product Label (5 copies).

In addition, three (3) copies of the following documents that include data/information in support of this application are being submitted:

- Product Analysis - This document provides a description of product identity and the manufacturing process for the component monophages and ECP-100 and includes data on sample analysis and physical/chemical properties.

- Safety Assessment – This document presents a review of the available safety information on bacteriophages and a waiver request for all microbial pesticide toxicology data requirements.

- Experimental Program – The experimental program that Intralytix is proposing for ECP-100 and a brief summary of the presumptive efficacy of ECP-100 are presented in this document.

- Tolerance Petition – Since the proposed use involves food-contact surfaces, Intralytix is submitting a tolerance petition that requests a temporary exemption for the requirement of a tolerance for ECP-100. The "Notice of Filing" will be submitted separately, by e-mail.

Intralytix believes that this submission is subject to PRIA Category B610 since the Biopesticides and Pollution Prevention Division (BPPD) will have primary responsibility for reviewing the data associated with this application and petition. The PRIA fee for Category B610 is $11,025. Since Intralytix qualifies for a 75% fee waiver, a payment of $2756.25 is being submitted. Supporting documentation for a fee waiver are enclosed.

If you have any questions about this submission, please contact me at (202) 393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com.

Sincerely,

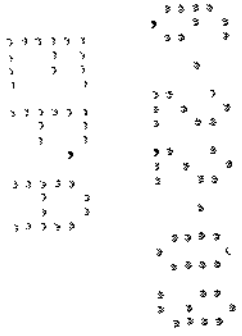
Eliot Harrison
Agent for Intralytix, Inc.

LEWIS &
HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

May 30, 2009

Velma Noble, Product Manager (31)
Regulatory Management Branch No. I
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202



re: **Application for Experimental Use Permit**
Product: ECP-100™
Active Ingredient: *E. coli* 0157:H7 Specific Bacteriophages
Applicant: Intralytix, Inc.
Data Transmittal Letter for Studies Supporting Experimental Use Permit

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am submitting three (3) copies of the following studies in support of the Experimental Use Permit (EUP) application for ECP-100:

- Volume 1 of 3
  ECP-100 – Product Identity, Manufacturing Process, Sample Deposition and Discussion of the Formation of Impurities (33 pg).
  MRID#

- Volume 2 of 3
  ECP-100 – Analysis of Samples, Certification of Limits, and Physical and Chemical Characteristics (13 pg).
  MRID#

- Volume 3 of 3
  Waiver Requests for Microbial Pesticide Toxicology Data Requirements and Discussion of Safety Issues (231 pg).
  MRID#

If you have any questions about this submission, please contact me at (202) 393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com.

Sincerely,

Eliot Harrison
Agent for Intralytix, Inc.

LEWIS &
HARRISON
Consultants in Government Affairs

122 C Street, N.W., Suite 740
Washington, D.C. 20001
telephone 202.393.3903
fax 202.393.3906

May 30, 2009

Velma Noble, Product Manager (31)
Regulatory Management Branch No. 1
Antimicrobials Division (7510P)
Office of Pesticide Programs
Environmental Protection Agency
One Potomac Yards
2777 S. Crystal Drive
Arlington, VA 22202

re: **Pesticide Petition Proposing to Amend 40 CFR §180.940(c)**
Microbial Pesticide: *E. coli* 0157:H7 Specific Bacteriophages
Petitioner: Intralytix, Inc.

Dear Ms. Noble:

On behalf of Intralytix, Inc., I am submitting a pesticide petition pursuant to Section 408(d) of the Federal Food, Drug and Cosmetic Act (""FFDCA") and 40 CFR §180.940 with respect to the microbial pesticide, *E. coli* 0157:H7 specific bacteriophages. The petition requests that the Agency amend 40 CFR §180.940(c) by establishing a temporary exemption from the requirement of a tolerance for the use of *E. coli* 0157:H7 specific bacteriophages on food-contact surfaces in food-processing plants.

Attached hereto, in triplicate or referenced, and constituting the petition are the following:

Section A: Identity of the Pesticide.

Section B: Use of the Pesticide.

Section C: Safety Issues Related to the Petition.

Section D: Residue Issues Related to the Petition.

Section E: Practicable Methods for Removing Residues that Exceed the Tolerance Level.

Section F: Proposed Tolerance.

Section G: Reasonable Grounds in Support of the Petiton.

An information summary ("Notice of Filing") for this petition, including arguments cited in support of the petition and a statement that the petitioner agrees that the summary may be published as part of the notice for the petition, is also attached.

If you have any questions about this petition, please contact me at (202)-393-3903, ext. 14 or by e-mail at eharrison@lewisharrison.com

Sincerely,

Eliot Harrison
Agent for Intralytix, Inc.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1999 – 452-584/25008

Form Approved. OMB No. 2070-0040.

United States
ENVIRONMENTAL PROTECTION AGENCY
Washington, DC 20460

OPP Identifier Number

JW 4:30

Dollie J Stein & Tess[?] Yarde[?] with 12/8/09 8/4/92

Office of Pesticides Programs (7505C)

Application for Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only

1. Type of Application

[X] New [ ] Amendment (See No. 2)

[ ] Extension *(Give Permit Number below)*

Permit Number

2. Briefly explain (attach a separate sheet if necessary)

3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be Issued (Include Zip Code) *(Type or Print)*

Intralytix, Inc.
323 W. Camden Street, Suite 675
Baltimore, MD 21210

4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (Include Zip Code) *(Type or Print)*

EPA Company Number 74234 – EUP – E

5. Name of Product

ECP-100

6. Is Product Registered with EPA?

[X] No

[ ] Yes (Give Registration Number or File Symbol below)

Registration Number ______

File Symbol ______

7. Total Quantity of Product Proposed for Shipment/Use

Pounds of formulated product 120,000

Pounds of active ingredient 0.392

8. Acreage or Area to be Treated

1.2 x 106 square feet

9. Proposed Period of Shipment/Use

September 1, 2009-
September 1, 2011

10. Places from which Shipped

701 E. Pratt Street, Baltimore, MD 21202

11. Crop/Site to be Treated

Food and Non-Food Contact Surfaces in food-processing plants

12. Specify the name and number of the contact person most familiar with this application.

Dr. Gary Pasternack, 410-659-7330

13. Signature of Applicant or Authorized Firm Representative

[signature]

14. Title Agent for Intralytix

15. Date Signed 5/30/09

Certification

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

Below for EPA Use Only

In any correspondence on this application, refer to this number

Received by:
EPA-OPP Registration Division,
Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number

EPA Form 8570-17 (Rev. 1-94) Previous Editions are Obsolete

FOR OFFICIAL USE ONLY

FILE SYMBOL	
REGISTRATION NO.	74234-EUP-E

CONFIDENTIAL STATEMENT OF FORMULA ENCLOSED

DATE SUBMITTED	SUBMITTED BY (✓)	
	APPLICANT	BASIC SUPPLIER
JUN 23 2009		

Do Not Write Comments, Formula, or Parts of Formula on This Envelope

NOTE

It shall be unlawful—for any person to use for his own advantage or to reveal, other than to the Secretary, or officials or employees of the United States Department of Agriculture or other Federal agencies, or to the courts in response to a subpoena, or to physicians, and in emergencies to pharmacists and other qualified persons, for use in the preparation of antidotes, in accordance with such directions as the Secretary may prescribe, any information relative to formulas of products acquired by authority of Section 4 of the "Federal Insecticide, Fungicide, and Rodenticide Act."

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Pages 224-225-*Confidential Statement of Formula may be entitled to confidential treatment*